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BIOSENSORS: A NOVEL DIAGNOSTIC APPROACH

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Abstract: The detection of pathogens is key in prevention and identification of diseases. Biosensors are analytical devices that allow detection of biomolecules in real time, highly specific and sensitive format and can essentially serves as low cost and highly efficient devices for diagnosis of pathogenic diseases. Different types of biosensors are being employed for detection of pathogenic microbes. Thus, diagnosis of pathogens by biosensors may become more popular than the standard methods, although achieving high sensitivity and specificity are still important challenges.

Keywords: Biosensors, Diagnosis, Disease, Pathogens.

Introduction

A biosensor is a compact analytical device with a ligand–specific biorecognition element, e.g. antibody, enzyme, receptor, nucleic acid, aptamers, peptide/ protein, lectin, cells, tissue or whole organisms, immobilized on a sensor surface integrated directly or indirectly with a signal conversion unit called transducer. The physiological interaction between the ligand and the biorecognition element is translated, by the transducer, into a measurable electric signal, which is further deciphered by a computer–aided readout system for the user (Arora *et al.,* 2010). Biosensors are chiefly classified based on the biorecognition element and the transducers.

Properties of a biosensor

i. ***Specificity*****:** A biosensor should be specific to the analyte which it interact.

ii. ***Durability*****:** It should withstand repeated usage.

iii. ***Independency*****:** It should not be affected by variations in the environment like temperature, pH etc.

iv. ***Stability*****:** The results produced by interaction should be corresponding to the concentration of analyte.

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v. ***Ease of use and transport:*** It should be small in size so that it can be easily carried and used.

Components and mechanism of a biosensor

A biosensor mainly consists of two parts

i. **Biorecognition/ Biological part:** This constitutes of enzymes antibodies etc., which mainly interacts with the analyte particles and induce a physical change in these particles.

ii. **Transducer part:** It collects information from the biological part, converts, amplifies and display them.

In order to form a biosensor, the biological particles are immobilized on the transducer surface which acts as a point of contact between the transducer and analyte. When a biosensor is used to analyse a sample, the biological part specific to the analyte molecules, interacts specifically and efficiently. This produces a physicochemical change of the transducer surface. This change is picked up by the transducer and gets converted into electric signals. These then undergo amplification, interpretation and finally display of these electric units accounting to the amount of analyte present in the sample.

Biorecognition Elements (Antibodies)

Due to the simplification of protein expression, peptide synthesis and purification processes, target antigen can be generated in large amounts for antibody production and characterization. Further development of molecular platforms and recombinant DNA technology has aided in selection of high affinity antibodies and tailoring it to desired characteristics. Availability of improved antibody purification methodologies has also contributed to antibody's increased utilization (Ayyar et al., 2012). Biorecognition is the key aspect in a biosensor design so it is very essential to choose the biorecognition probe carefully. Antibodies are undoubtedly the most popular class of biorecognition probes owing to their high binding affinity (Connelly and Baeumner, 2012) and explicit target specificity. These characteristics contribute to the sensitivity and specificity of the biosensor to detect the target in complex sample matrices. Antibodies or immunoglobulins are Y–shaped proteins produced by B–cell as host's immune response to counter antigen. Antibodies bind the target antigen and destroy it in order to protect the host. For biosensor application, a suitable host is immunized with the antigen of interest along with the adjuvant to achieve a specific antibody response against the target. Antibodies used as probes can be polyclonal, monoclonal or recombinant (Ayyar et al., 2012).

i. **Polyclonal antibodies (pAbs):** Polyclonal antibodies (pAbs) are a mixture of antibodies produced against highly immunogenic regions of the antigen with different epitope specificities and clonal affinities, produced by the B cell population of the host in response to an antigen. Large animals such as rabbit, goat and sheep are preferred for pAb generation due to the volume of blood that can be drawn leading to the increased concentration of pAbs harvested from the animal serum. pAb generation is less time consuming and offers the advantages of being stable. pAbs are very useful in detecting similar antigens, contributed by multitude of antibodies obtained against various epitopes of the antigen. However, there are issues of batch variation, lack of high affinity and singular epitope specificity (Ayyar et al., 2012), which can be essential in some of the biosensor applications.

ii. **Monoclonal antibodies (mAbs):** Monoclonal antibodies (mAbs) are obtained by fusion of antibody producing B–cells with the immortal myeloma cells, which leads to the generation of hybrid cells called 'hybridoma'. Hybridomas retain the antibody–producing feature of B–cells and the immortality processed by the myeloma cells. Each B–cell produces antibody against a single epitope of the antigen, which after rigorous selection and cloning–out procedure are isolated and antibodies are produced from hybridoma cells with single clonal composition. Murine hosts are commonly used for mAb generation, however, there are reports of using rats and rabbits for this purpose. mAbs offers the advantages of being reproducible, large quantity generation, homogeneous composition with high epitope specificity and binding affinity. However, mAb production is expensive and time consuming compared to pAbs and its development requires considerable skills.

iii. **Recombinant antibodies (rAbs):** Recombinant antibodies (rAbs) are antibodies or antibody fragments generated *in vitro* using molecular techniques. Recombinant antibodies are made by combining the inherent property of immune system along with random recombination of VH and VL, to generate of a vast library of antibodies, which is further screened for specific binders (Welbeck et al., 2011). rAb generation and isolation is usually time consuming, however, development of large diverse libraries using synthetic and semisynthetic approaches has made it possible to isolate rAbs against various target without going through the tedious immunization and library construction procedures, thus saving time and resources. They are advantageous compared to pAbs and mAbs due to the fact that by employing combinatorial recombinant technologies large library of antibodies can be generated. Antigen specific–selection is done by using molecular display platforms (phage display, ribosome display and yeast display) and further screening based on binding kinetics

can be carried out through high–throughput methods (Saerens et al., 2008). rAbs are amenable to engineering for improving its characteristics such as binding, stability, purification and specificity along with the feasibility of format optimization. As biosensor probes they may serve as a better alternative to full length antibodies due to their small size that can help in high density immobilization and reduced non–specific binding due to the absence of Fc fragment (Saerens et al., 2008).

Transducers

A transducer converts the biorecognition event into a measurable signal. Many different types of transducers have been described, however, most of them can be classified as electrochemical, optical, mass–based and calorimetric with the first three being the commonly employed ones for pathogen detection (Monošík et al., 2012).

Electrochemical biosensors: Electrochemical biosensors measure the change in electrical properties following biorecognition, as a result of production or consumption of the ions or electrons (Mohanty and Kougianos, 2006). Electrochemical biosensors are further classified into:

i. **Amperometric biosensors**: They measure the generated current at a constant potential and are the most commonly used class of electrochemical biosensors (Velusamy et al., 2010). A variant of amperometric biosensors are voltametric biosensors that measure electrical current during controlled variations of the potential.

ii. **Potentiometric biosensors**: They measure difference in potential (Velusamy et al., 2010).

iii. **Impedimetric / conductometric biosensors:** They function by measuring the change in electrical resistance/ conductance of the solution (Mohanty and Kougianos, 2006).

iv. **Optical biosensors:** They measure changes in intensity of light. Detection elements in such biosensors are frequently based on luminescence, fluorescence, phosphorescence, colorimetry, reflectance, light polarization and rotation, interference, spectroscopy, ellipsometry and surface plasmon resonance (SPR) (Luong et al., 2008; Velusamy et al., 2010; Huang et al., 2011). However, fluorescence and SPR–based biosensors are most common (Velusamy et al., 2010).

v. **Mass–based biosensors**: They detect a change in mass that occurs following the interaction between the biorecognition element and the target analyte (Mohanty and Kougianos, 2006; Holford et al., 2012). Such sensors generally use piezoelectric materials that change their resonant frequency, following the change in mass, generating acoustic

waves. The travelling wave either propagates along the surface of the substrate (surface acoustic wave, SAW) or through the surface of the substrate (bulk acoustic wave, BAW) (Rocha–Gaso et al., 2009). The most commonly used piezoelectric biosensors employ Quartz Crystal Microbalance (QCM) (Holford et al., 2012) and is based on bulk acoustic wave propagation.

Immobilization of Antibodies to the Sensor Surface

Apart from selecting suitable antibodies for probing, a critical factor that influences biosensing mechanism is the immobilization of the antibodies to the sensor surface, which in turn depends on the properties of the sensor interface. Various types of sensor surfaces have been studied (gold, silver, glass, platinum, silica) for this purpose. Ideally sensor surface should be stable, providing large surface area for high density probe immobilization, possessing excellent electrical and thermal conductivity, low diffusion rates, and less signal–to–noise ratio due to matrix effects (Holford et al., 2012). It is hard to get all the desired characteristics on one surface and still keep it small for the device to be portable. Advent of high performance matrices such as carbon nanotubes, fabricated nanoparticles, self–assembled monolayers (SAMs) and quantum dots has led to the development of new generation sensor platforms compatible with the aforementioned sensor needs (Holford et al., 2012). The antibodies can be coupled to the sensor surface by various methods. Passive absorption, covalent coupling, matrix entrapment, encapsulation and affinity tags are the most commonly used methods with their own pros and cons.

Passive Absorption

Physical absorption is a simple process in which the antibodies attach to the sensor surface by Van der Waals, hydrogen or hydrophobic interactions or a combination of all. This process requires minimal antibody manipulation, leaving the antibody unmodified, achieving a high immobilization level. However, as the antibodies are attached by weak interactions, such surfaces have issues of antibody leakage, random antibody orientation, uneven distribution and no tolerance to surface regeneration (Saerens et al., 2008).

Covalent Coupling

Covalent coupling is the most commonly employed method for antibody immobilization to the sensor surfaces. These surfaces are available commercially or can be chemically activated by using glutaraldehyde, periodate, carbodiimide and maleinimides succinimide esters to crosslink the antibodies. Covalent coupling provides a uniform and a stable surface immune to issues like aggregation or antibody leaching over time or regeneration procedures.

Conversely, the immobilization requires the manipulation of antibodies, which may render it non–functional or may even degrade it in the process. This can be avoided by protecting the antigen binding region in the antibody before modification (Yoon et al., 2011). General covalent coupling procedures do not ensure proper orientation of the antibodies, which is important for antigen binding and in turn biosensor sensitivity. There are many ways to overcome this issue for e.g. identifying free thiol groups, farther from the active site, or engineering one in the antibodies can help in directional orientation of the antibodies on the gold surface either directly or through a linker or using an intermediate layer such as protein A or G which can be covalently coupled to the surface (Makaraviciute and Ramanaviciene, 2013).

Matrix Entrapment

Matrix entrapment relies on trapping the antibody into a polymeric gel matrix and then immobilizing to the sensor surface. The matrices are thin and porous to allow antigen–antibody interaction. Most commonly used matrices are starch, cellulose, alginate, polyacrylamide, polycarbonate, polyurethane and silica gel. This is a simple and a reliable method of antibody immobilization generating a stable surface, however, it is necessary to ensure that the used matrix is compatible with the sensor surface and will not interfere with antibody interaction (Gupta and Chaudhury, 2007; Monošík et al., 2012).

Affinity Tags

Mostly recombinant antibodies are expressed by conjugating it genetically with peptides / proteins, which acts as an affinity tag, which facilitates its purification and detection. This immobilization technique is based on covalent immobilization of a specific binding partner to the affinity tag on to the sensor surface, creating a stable surface. The chimeric antibody, to be used as biorecognition probe, is passed over the sensor surface causing a directional orientation of the antibody due to affinity tag. Such immobilization procedure requires less/no antibody modification, mild regeneration conditions and the amount of the probe immobilized on the surface can be controlled. This method keeps the binding regions of the probe free and very beneficial in the regard that crude lysate containing antibody can be used, as only specific antibody will be immobilized (Ayyar et al., 2010).

Conclusions

Advances in nanotechnology and microfluidics have allowed miniaturization and subsequently, development of point–of–care and lab–on–a–chip diagnostics. This has revolutionized the field of biosensing and thus, there is increased interest in biosensors.

However, veterinary pathogen detection is still to harness this technology to its benefit with biosensing currently finding use chiefly in mastitis detection. Viral veterinary diagnostics can greatly benefit from biosensors allowing rapid, robust cheap and simple alternatives to conventional viral detection methodologies. In addition, biosensors allow “on–site” testing, and can be performed and interpreted, within a matter of seconds or minutes, by farmers or veterinarians. This is a lucrative preposition compared to collection and shipment of samples followed by waiting for weeks to get results. Consequently, biosensors can allow veterinarians to provide specific and timely treatment to animals and thus, reducing the resulting morbidity and mortality. Additionally, it allows checking the spread of contagious pathogens to another animals and humans (in case of zoonotic pathogens) However, none of the biosensors for veterinary virus detection has made to market yet and it can be rightly concluded that biosensing for veterinary pathogens is still in its infancy. It needs to be seen if the global technology push coupled with the inevitability of cheap, rapid and “on–site” diagnostics in veterinary sector will instigate an upsurge of interest in biosensor development for veterinary pathogen detection.

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