Journal of Analysis and Computation (JAC)
(An International Peer Reviewed Journal), www.ijaconline.com, ISSN 0973-2861
Volume XVII, Issue II, July-Dec. 2023

DEVELOPMENT OF OPTICAL BIOSENSORS FOR DIAGNOSIS OF AMYLOID- B PEPTIDE AND GLIAL FIBRILLARY ACIDIC PROTEIN

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ABSTRACT

Currently, the use of biosensors is crucial in diagnosis, monitoring progression of a disease, food safety standards, drug development and biomedical research. Physiological changes of any medical state or pathogenic process altered during disease progression are characterized by measurable indicators known as biomarkers. Neurological Disorders pose a great burden on global population because of progressive deterioration of neuronal cells such as in Alzheimer's Disease (AD). AD is major contributor of dementia diagnosed by certain biomarkers as Amyloid-β (Aβ) peptide and Glial Fibrillary Acidic Protein (GFAP) levels in Cerebrospinal Fluid (CSF) and Blood plasma. Therefore, it is cardinal to understand some powerful analytical tools which have the capacity to identify even the smallest physiological change in sufferers' body in the level of Aβ with great sensitivity. Since, collection of CSF via lumbar puncture is quite uncomfortable so blood based biosensing is an alternate approach for quantification, to overcome the drawbacks of routine screening programs. The detection limit of Aβ peptide and GFAP has been found in the range 2.4-3.6 pg/ml and 0.2-0.5pg/ml of plasma respectively. It is believed that optical biosensing of blood-based biomarkers of the AD in the early diagnosis of AD will prove a milestone and give a jump to the disease therapeutics.

Keywords- Alzheimer's Disease, Amyloid-β peptide, Biomarker, Biosensor, GFAP, Optical Biosensor.

1. INTRODUCTION

Globally, prolonged neurological disorders are affecting a large population with old age, which ultimately leads to dementia. Dementia is characterized by cognitive decline, memory impairments, behavioral changes which severely affecting a person's ability to live independently. Most common cases of dementia is of AD estimating 60-70% which has an advancing global ubiquity without any cure [1].

AD, most common cause of dementia and all other neurological disorders, is the leading cause of death globally. Preclinical, AD is diagnosed by neuropathological changes like shrinkage of brain tissue known as brain atrophy of the entorhinal cortex of temporal lobe considered as negative lesions [2, 3, 4]. Furthermore, it is characterized by disability of a person in doing day to day activities like walking and talking because of cognitive disability and language problem, prevalent long before memory disorders. Gradual progressive loss of memory is combined with inability to acquire new information with an impaired recording,

storage and retrieval of information. Due to which sufferer requires continuous personal care. Therefore, AD is a multifactorial and heterogeneous disease with major pathological changes with differing symptoms in different sufferers' [5]. The hallmarks of AD are accumulation of extracellular Aβ peptides in the form of plaques and hyperphosphorylated tau protein intracellularly in the form of neurofibrillary tangles in the hippocampal region and cerebral cortex as positive lesions. These regions of brain are mainly involved in memory and navigation [6, 7]. This deposition interferes with the synapse functioning and neurotransmitters release accompanied by loss of connections and death of neuronal cells. Another characterizing feature of AD is over-activation of microglial cells (microgliasis) and astrocytes (astrogliasis). These are phagocytic immune cells which helps in tissue repairing and defense mechanism. These also help in removal of unwanted synapses and neuronal cells by engulfing and clearing the Central Nervous System (CNS) debris. In AD these cells get accumulated near Aβ fibrils and generates a chronic inflammatory response by reacting to the cytokines produced by microglial cells in the brain tissue [8, 9].

Since, various biomarkers are there for the diagnosis of neurological disorders like in AD Aβ42 peptide, total τ protein, GFAP, microRNAs present in CSF and blood plasma with certain detection limit can be found useful for the early diagnosis of AD before the memory disorders as there is no permanent cure but early detection can help in its delay [10, 11, 12].

2. BIOMARKERS

A biomarker or biological marker is a quantifiable biological indicators of any state or disease in biomedical applications, capturing what is happening in an organism or cell at that particular time. While examining any typical pathogenic processes, biological processes or pharmacologic reactions, these biomarkers are examined and accessed using blood, urine etc., hence used in many scientific fields [13]. Today, a variety of biomarkers are employed which are simple to test and hence included in simple medical examinations. Each physiological system have its certain specific biomarkers which are used to predict the seriousness of concerned disease. There are different types of biomarkers like molecular biomarkers which measures molecules in biological samples (serum, plasma, CSF) radiographic which are obtained from imaging studies, histographic reflecting histological changes in cell or tissue and physiological biomarkers measuring body processes [8,10]. Over the past years, CSF and Positron Emission Tomography (PET) biomarkers have influenced the drug design and neurodegeneration researches [15]. CSF Aβ42, total tau and phosphorylated tau protein are well known validated for AD specificity and sensitivity [16]. With the development of new generation of immunological assays during the past decade, it is now possible to quantify protein biomarkers from blood samples, providing fresh insights into the area of CNS derived biomarkers. The research on the use of blood GFAP as a biomarker is also expanding as well adding to the wealth of information. The most common neurodegenerative diseases such as AD, Frontotemporal degeneration, prion disease and Parkinson's Disease have been linked to higher CSF GFAP levels [17, 18, 19, 20].

The most prevalent cell type present in human CNS, astrocytes, contains the main intermediatory filament (cytoskeletal protein) known as GFAP. It contributes to the integrity of Blood-Brain Barrier (BBB), architecture of white matter, astrocytic formation and motility with myelination of neurons. Extracerebral sources of this protein have not been discovered, considering it to be very brain-specific consequence to very low levels in blood stream of healthy individuals. GFAP release in peripheral blood is thought to be because of disruption of BBB or loss of astrocytic structural integrity [19, 20, 21, 22].

3. SENSORS

Today, we benefit from the science and technological advancements that makes our lives better [23]. We rely on numerous devices that help us interact with the physical world which are further rely on sensors to function. A sensor is described as a module that assist in detecting changes in physical or electrical quantity, converting them into signals that can be easily monitored or analyzed. The ability to correctly track processes that have an influence of person's nature and to detect even the smallest physiological change in the body with a great sensitivity has resulted in a significant increase in life quality.

A biosensor is a type of analytical tool that produces a quantifiable signal by combining electrical component with a biological element like an antibody or enzyme. They are self-sufficient integrated machines whose main function is to swiftly deliver accurate and trustworthy information about the targeted analyte in real time. The electrical component can identify a physiological change or the presence of different chemicals or compounds in the sample even at very low concentrations. The development of biosensors may use a wide variety of methodologies [24].

3.1 Biosensor design and working: A typical design of biosensor consists of 5 main components [24, 25]:

Analyte: A substance of interest that needs to be detected like glucose, ammonia alcohol etc.

Bioreceptor: A biological detector element that can specifically sense or recognize the target element or analyte by a process called biorecognition. This can produce a signal in the form of heat, pH change, light etc. these biological receptors can be antibody, DNA, RNA, aptamer, cell receptor, cell, tissue or organelle which directly or indirectly identify the target substances. These can be grouped into two categories: catalytic and non-catalytic. Devices designed for continuous monitoring of chemicals check at millimolar or micromolar concentrations utilize the catalytic group of biological receptors having tissues or enzymes [16]. In a catalytic biosensor, the interaction between the analyte and the bioreceptor leads to the production of a new biochemical reaction product. While non-catalytic group is mostly utilized in biosensors to assess analytes like steroids, pharmaceuticals often exist at extremely low concentrations (micro-picomolar range). The analyte is irrevocably attached to the receptor, and no new biochemical reaction product is created as a result of the contact. Receptors which include antibody, nucleic acid can be used only once and thereafter discarded [26].

Transducer: Another key component of any biosensor having capability to convert one form of energy to another by a process called signalization. When analyte interacts with the biological receptor it generates a biochemical signal which is received and converted by transducer into quantifiable signal. Transducers either generate electrochemical or optical signal proportionate to the interactions occurred.

Electronics: Comprises of a signal processing unit in which the signal obtained from transducer be amplified and processed to be converted from analog to digital form. This digitally acquired signal is ready to quantify by the display unit.

Display: The displayed signals are understandable by the user interpretation system like computer which generates the output. The output signal can be in the form of image, graph or table to read or understand.

3.2 Important characteristics of biosensors:

Selectivity: A receptor's selectivity refers to the capacity to identify a particular analyte in a sample that contains various molecules and unwanted pollutants. Best illustration of it is antigen-antibody interaction. Selectivity is a crucial component, particularly in medical applications, often where the test sample is urine or blood, includes a large number of molecules in completion with the desired target molecule to interact with the bioreceptor [23, 24, 25].

Detection limit or sensitivity: the lowest achievable concentration of the target that can produce a quantifiable signal is detection limit or limit of detection (LOD). These are used mainly in environmental monitoring and medical applications where the concentration of the analyte is in ng/ml or fg/ml with a great sensitivity [23, 24, 25].

Stability: One of the essential qualities in biosensor applications where ongoing monitoring is necessary is stability. Stability is the degree to which the biosensing equipment is vulnerable to environmental perturbations both inside and outside. Such interruptions might result in inaccurate output signals during measurements, which would compromise the biosensor device's precision and accuracy. As transducers and other electronic components are primarily temperature sensitive, which can negatively affect their stability and tend to deteriorate receptors integrity due to temperature changes [23, 24, 25].

Response time: This characteristic controls how quickly the target analyte interacts with the biological receptor in the biosensor to produce 95% of the results [23, 24, 25].

Reproducibility: The biosensor's reproducibility refers to its capacity to provide the same results under identical testing conditions. The transducer in a biosensor are precise and accurate, which defines repeatability. When a sample is tested more than once, accuracy refers to the sensor's capability to offer a mean value that is near to the real value while precision refers to the sensor's ability to produce identical findings every time. Reliable and strong inferences about a biosensor's reaction are made possible by reproducible signals [23, 24, 25].

Range or linearity: When a series of measurements are made at various concentrations, the accuracy of the signal is determined by the biosensor's linearity. This characteristic provides information on the biosensor's resolution, which is the smallest change in the concentration of the target analyte required to trigger a response. The substrate concentration may be detected at higher levels when linearity (straight line) is increased [23, 24, 25].

3.3 Applications of biosensors: For a variety of applications, a variety of electrochemical, optical, and acoustic sensing methods have been used in conjunction with their integration into analytical instruments. Figure 1 displays the various study fields where biosensors have been employed [24, 25, 27].

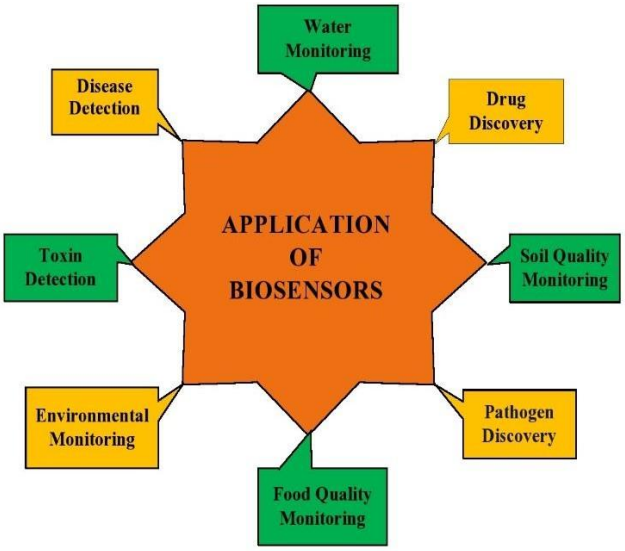

Fig.1 Major Areas of use of biosensors

3.4 Classification of biosensors: The subject of classification of biosensors is broad and multifaceted. There are different criteria to categorize biosensors, and Figure 2 depicts the general categorization method, but the two that are most frequently used are the biorecognition component and the signal transduction component [28].

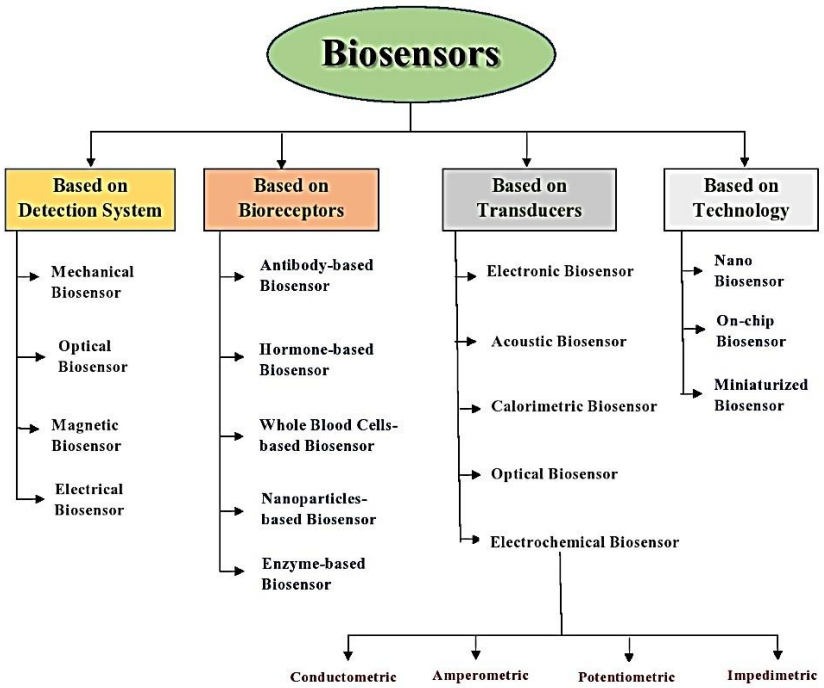

Fig.2 Flowchart of classification of biosensors on various basis

4. OPTICAL BIOSENSORS

Analytical technology for the identification of biological and chemical species is rapidly being influenced by optical biosensors. Optical biosensors are a viable replacement for traditional analytical methods because of their high specification, sensitivity, compact size, and low cost. These are the analytical tools that uses light to detect any quantifiable biological recation. They are extensively utilized in many different industries, including as food safety,

environmental monitoring, and medical diagnostics offering numerous benefits like rapid response, high sensitivity and the potential for miniaturization. The basic principle behind optical biosensors involves the interaction of light with the biorecognized element like antibodies, enzymes or DNA probes that binds to the target analyte. As a result of this interaction, an optical signal that may be detected is proportional to the target analyte's concentration [29, 30, 31, 32].

4.1 Types of optical biosensors based on use of transducer system: There are different types of optical biosensors, but some common ones include:

1) *Surface Plasmon Resonance (SPR) Biosensors:* SPR biosensors use the phenomenon of SPR, which occurs when polarized light interacts with a thin metal film. Binding events on the sensor surface cause a change in the refractive index and lead to a shift in the resonance angle, which can be detected and correlated with the analyte concentration.

2) *Fluorescence-based Biosensors:* These biosensors use fluorescent molecules that emit light when excited by a specific wavelength. The binding of the analyte to the biorecognition element can cause a change in the fluorescence signal, allowing for detection and quantification. Because of its great selectivity, sensitivity, and quick reaction time, this type of biosensor is thoroughly researched for use in medical diagnostics, environmental and monitoring food quality applications, and other applications. This biosensor makes use of a variety of fluorescent dyes, including QDs, dyes, and fluorescent proteins.

3) *Luminescence-based Biosensors*: Luminescent biosensors use various luminescent materials, such as quantum dots or upconversion nanoparticles, to generate a measurable signal. The presence of the target analyte leads to a change in the luminescence properties, which can be detected optically. Chemiluminescence-based biosensors have attracted a lot of interest due to their ease of use, low detection limit, wide calibration limit, and inexpensive apparatus.

4) *Fiber Optic Biosensors:* Fiber optic biosensors utilize optical fibers as the sensing platform. The fibers can be modified with recognition elements, and the interaction between the analyte and the recognition element alters the light transmission through the fiber. This change in light intensity or wavelength can be detected and correlated with the analyte concentration.

5) *Evanescent Wave Biosensors*: Evanescent wave biosensors exploit the phenomenon of total internal reflection, where light propagates along a waveguide. The sensing region of the waveguide is coated with a biorecognition element, and when the target analyte binds to it, the refractive index changes and affects the propagation of the light, allowing for detection.

4.2 Device architecture and working: Typically, an enzyme system is used for the detection, which catalytically changes the analytes into products that may be reduced or oxidized at a working electrode that is kept at a certain voltage. This optical transducer's primary benefits are its inexpensive price and usage of biodegradable electrodes. An appropriate optical substrate is used to immobilize the detection of specific binding of the target analyte to the

corresponding optical biorecognition element. Optical sensors are sensitive and selective instruments for the in situ and real-time monitoring of molecular interactions as well as the detection of very low amounts of biological substances and chemicals [33, 34].

5. AD BIOMARKER INTERACTION IN OPTICAL BIOSENSORS

Structural changes of AD have been accessed by Magnetic Resonance Imaging (MRI) early than clinical changes. These preclinical changes cannot be diagnosed by clinical measurements thus, biomarkers are required to detect patient's crucial stage of illness and for the persons who are more likely to acquire the condition [35]. In-vivo diagnosis of AD led to a new paradigm which is based on biomarkers detection in CSF or via PET scans of brain tissue. Both the diagnostic methods are complex associated with high costs and invasiveness, as CSF is collected via lumbar puncture restricting the implementation of CSF based biomarkers in common practice [36]. In contrast to this, blood-based biomarkers for initial clinical diagnosis can be proved a good alternative approach with less invasive and cost effective nature tool for clinical trials and also population screening does not need to be stressed [20, 35]. But there are several difficulties associated with detecting brain disorder biomarkers in blood as BBB could prevent free movement of these chemicals between CNS and blood compartments. Often these will find in very low concentrations in blood which necessitates sensitive and accurate assays with thorough validation studies. So there should be some targeted blood-based biomarkers like plasma Aβ, plasma tau protein, plasma neurofibrillary light chains and certain proteins associated with AD [37, 38]. Well characterized therapeutic compounds as biochemical markers are needed to diagnose AD in its early stage to slow down its progression.

By utilizing various optical techniques such as SPR, interferometry, fluorescence, and Raman spectroscopy, researchers have successfully developed biosensors capable of detecting and quantifying Aβ and GFAP with high sensitivity and specificity [36, 39]. These biosensors can detect biomarkers at low concentrations in complex biological samples, enabling early disease detection and disease progression monitoring. Furthermore, the development of miniaturized and portable optical biosensors has the potential to revolutionize point-of-care diagnostics, bringing rapid and accurate testing directly to the patient. This advancement in technology could lead to early intervention, personalized treatment strategies, and improved patient outcomes.

It is clear from the seemingly endless literature on biosensors over the past few decades that the technology is appealing to both industry and academics (Table 1). Biosensor technology makes use of the unique properties of a biological recognition event on a transducing device. To create novel biosensors with applications, there are several approaches to merge biology, chemistry, physics, mathematics, and engineering. Here we can merge AD blood plasma biomarkers as a target molecule to detect and diagnose AD in early stages with the optical biosensors. Immobilized antibodies of these protein molecules will be used as bioreceptor which interacts with the specific Aβ peptides and GFAP. These immobilized molecules will be tagged with the fluorescent molecules or dyes and when it interacts with the antigen these can produce the fluorescence. When these fluorescent tagged molecules get light, transducer system based on interaction generates the signal in the form of visual signal. In this situation, the analyte and the bioreceptor interaction is transformed into an appropriate output that the user can understand [32, 40, 41, 42, 43].

Table 1. Summary of various biosensors used for neurodegenerative disorders markers

S.no.	Biosensor	Mechanism	Limit of detection of biomarkers	Reference
1.	MircoRNA based nanobiosensors	Colorimetric detection of gold nanoparticles and hybridization chain reaction amplified miR-137	0.25 nm of miR-137 in blood sample	[44]
2.	Ultrasensitive electrochemical biosensor	Carbon electrodes fabricated with electrochemically reduced graphene oxide with gold nanowires	1.7 fM of miR-137 in serum	[45]
3.	Label-free optical nanosensors	Four sensors made up of anodic aluminium oxide (AAO) nanopore thin film and mounted on a glass substrate to make a sensor chip. Antibodies are immbolised on this sensor chip	7.8 pg/ml and 15.6 pg/ml of Aβ and total τ protein respectively.	[46]
4.	Surface Enhanced Raman-Spectroscopy (SERS) based nanobiosensor	Multibranched nanopillar surfaces fabricated using polycarbonate on anodized aluminioum oxide molds with thin layer of Au evaporation, enabling them to detect thioflavin T	0.5 pg/ml.	[47]
5.	Electrochemical biosensor	Graphene oxide gold nanoparticles hydrogel electrode immobilised with thiolated cellular prion protein peptide probe	0.1 pm of Aβ in CSF or blood plasma	[48]
6.	Optical and Electrochemical biosensors	Ferrocene-encapsulated Zn zeolitic imidazole framework from Zn ions and 2-methylimidazole	0.5 μm of aβ in blood sample	[49]
7.	Electrochemical based single use meurobiosensor	11-amino-1- undecanethiol (11-aut)-modified polyethylene terephthalate coated indium tin oxide (ITO-pet) electrode was formed using multiwalled carbon gold-nanoparticle.	0.5 fg/ml in CSF and saliva	[50]
8.	Fluorescent Aptasensor and Colorimetric Aptablot	Carbon-dot based nitrogen doped aptasensor and Cu enhanced Au aptablot	3.64 ng/ml and 4.71 pg/ml of p-tau231 respectively in CSF and serum samples	[51]
9.	Graphene Field Effect Transistor biosensor	On-chip graphene field sensors directly detect GFAP from sample	20 fg/ml of GFAP in plasma samples	[22]
10.	Electrochemical and Surface Enhanced Raman Scattering based immunosensors	Acoustofluidic multimodal sensors and ZnO nanoarrays fabricated electrodes coated with Ag nanoparticles	62.3 fg/ml of Aβ in plasma samples	[52]

6. CHALLENGES AND FUTURE OUTLOOK

The speed, immunity to magnetic or electrical interference, and possibility for better information content of optical biosensors are beneficial, however the primary drawback could be the expensive cost of such equipment. Although optical biosensor production presents a number of technological challenges, additional study is still required to identify more effective substitutes. Some of them are as follows:

- The most challenging challenge in the creation of an optical biosensor is immobilization of biomaterials. During the process of immobilizing biomolecules on a solid substrate, material losses are seen.
- Contamination is a significant issue with biomolecules and the chemicals used in biosensors because of their seeping out. Biomolecules should be firmly connected to the transducer to prevent contamination.
- To identify a wide range of biomaterials, the selection range should be substantial.
- To combat many medical and technical issues, it is necessary to create low-cost optical biosensors for wide-scale application.

When designing innovative optical biosensors for practical applications, all scientific and practical factors, such as robustness, reproducibility, shelf life, and simplicity should be carefully taken into consideration. The optical detection method makes it feasible to build sensitive, low-cost analytical devices with a wide range of potential uses in portable biosensor systems, which poses the major challenge for future innovation and research in the field of optical biosensors.

7. CONCLUSION

In conclusion, the development of optical biosensors for the diagnosis of Aβ and GFAP represents a significant advancement in the field of biomedical diagnostics. Optical biosensors offer several advantages, including high sensitivity, real-time monitoring, and non-invasiveness, making them promising tools for the early detection and monitoring of neurodegenerative diseases such as AD. The detection of Aβ and GFAP, which are key biomarkers associated with AD and other neurological disorders, is crucial for accurate diagnosis and disease management. Traditional diagnostic methods often rely on invasive procedures or time-consuming laboratory tests. However, optical biosensors provide a more efficient and patient-friendly approach by leveraging the interaction between light and biological molecules. Continued research and collaboration between scientists, engineers, and medical professionals are essential for further refining these biosensors and translating them into clinical practice, ultimately leading to improved patient care and a better understanding of neurological disorders.

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