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Nanoarchitectured Bioconjugates and Bioreceptors Mediated Surface Plasmon Resonance Biosensor for *In Vitro* Diagnosis of Alzheimer's Disease: Development and Future Prospects

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ABSTRACT

Alzheimer's disease (AD) is an obvious neurological disorder characterized by progressive brain cell death that resulted in memory loss, cognitive decline, and finally dementia. Besides, AD is also affected by a multifunctional pathway, which leads to alteration in the biomolecular level as AD steps forward. Notwithstanding numerous diagnosis techniques, the conventionally engaged technology permits the detection of AD biomarkers with low sensitivity and poor selectivity. Concerning this, in recent years bioconjugates and bioreceptors based AD biomarkers recognition is gaining huge prospective to improved selectivity and sensitivity of AD at the molecular level. The present review deals with the recent progress in bioreceptors and bioconjugates mediated surface plasmon resonance (SPR) biosensor for *in vitro* diagnosis of AD. Fascinatingly, this review inculcates the information of assorted important AD biomarkers viz. beta-amyloid (Aβ), Tau protein, apolipoprotein (apoE4), 17-β-hydroxysteroid dehydrogenase type 10 (17β-HSD-10), acetylcholine, etc. In addition, this review sheds light on the utmost and unique methods of bioconjugates synthesis, which is holding the huge attention of researchers for AD biomarker detection and contributed to the development of simplistic, rapid, and socioeconomic sensitivity enhancement methods. Concisely, this review gives insight into the analytical performance of nanoarchitectured bioconjugate and bioreceptor-mediated SPR biosensor and their revolutionary benefits in terms of selectivity and sensitivity for *in vitro* diagnosis of AD biomarkers. Overall, this review gives a detailed overview of research done to date in the meadow of SPR biosensors in the *in vitro* diagnosis of AD, which paves the new pathway for futuristic biomedical applications.

KEYWORDS

Alzheimer's disease; surface plasmon resonance; bioconjugates; bioreceptors; *in vitro* diagnosis

HIGHLIGHTS

- AD recent updates and its biomarkers reviewed.
- There is no leading technology to rapidly sense and monitor AD.
- Bioconjugates as potential biosensing elements.
- Conjugation methods to link bioreceptors to nanomaterials have been highlighted.
- Role of bioconjugates and bioreceptors in AD biosensing through SPR biosensor have been discussed.

GRAPHICAL ABSTRACT

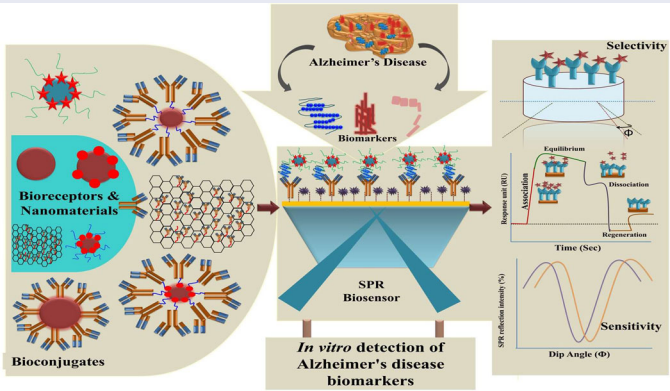

Figure 1. Nano-architectured bioconjugates and bioreceptors mediated SPR biosensor for highly sensitive and selective in vitro diagnosis of AD biomarkers.

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Introduction

Alzheimer disease

In recent years, the prevalence of neurodegenerative disorders (Alzheimer disease [AD], Parkinsonism disease, etc) has risen in the booming aging population. AD is among these diseases that remain un-curable as the key cause in the elderly owing to the limits of our current awareness.[1] As per historical data, in 1910, AD was a term introduced by Dr. Emil Kraepelin.[2] After a detailed finding of his patient, Auguste Deter brain abnormalities, Dr. Aloysius Alzheimer (German neuropathologist) identified the AD pathology for the first time on 3 November 1906.[3] Broadly, AD is a condition that leads to memory loss and eventual dementia triggered by the death of brain cells. Unfortunately, AD is an unavoidable neurologic disorder.[4] More precisely, AD is aggressive dementia, which leads to deficiencies in memory, communications, and normal behaviors of individuals.[5] Generally, AD is one of the catastrophic conditions for neuropathology, which mainly affects the older population over the age of 65.[6,7] In this way, 10% of individuals aged 65 years and 50% over the age of 85 years are affected due to AD. Whereas, AD in the United States is the fourth-largest cause of death and is widespread in many other nations. In 2018, official death certificates (United States) recorded 122,019 deaths from AD. Death from stroke, heart disease, and human immunodeficiency virus (HIV) declined between 2000 and 2018, while AD recorded deaths rose by 146.2%.[7] The predicated global prevalence would be quadruple by 114 million patients over the upcoming few decades by 2050, according to the World Health Organization.[8] This will noticeably contribute not only to a significant social impact but also to a higher cost to the health services around the world.[9] An annual (2010) global dementia burden of US$818 billion is projected due to the 46.8 million AD peoples worldwide.[10] The overall estimated care expense is about $100 and about $4 million for AD patients in the United States only.[11] The estimated cost of caring for the AD people will hit some US $2 trillion by 2030 and the total count of AD people will be 74.7 million.[12] In 2019, more than 16 million family members and other unpaid caregivers gave an estimated 18.6 billion hrs of treatment to people with AD and other diseases. While his treatment is worth almost $244 billion, the expense goes back to an increased risk of emotional distress and adverse outcomes for the mental and physical health of individuals. In America, total health insurance premiums, long-term care programs, and hospital programs in the year 2020 are forecast at $305 billion for people aged 65 or older with dementia.[7] Owing to this, from its inception, AD playing a vital role in the scientific field, which is evident from the continuous increase in the number of publications as well as methodologies developed till 2020, a total of 163,075 scientific papers are published as per PubMed search database. Plentiful literature mentioned that clinical diagnosis plays a key role in the early prognosis and diagnosis of AD. Additionally, modern medicines are based on biomolecular diagnostic knowledge, including pathology detection and diagnosis, the description of its burden, and the option of better pharmacological treatment.[13] Notwithstanding all medical findings, there are currently no viable methods that existed to prevent/cure AD at an early stage.[12] Besides, we witness that the AD progresses gradually and can continue for decades. Overall, AD is one of our times most critical health issues. Therefore, novel therapeutic approaches have to be implemented urgently to effectively delay the progression of AD.[1] Additionally, over the last century, because of major admirable changes in global health, life expectance has significantly increased. Ultimately, this can also increase the prevalence of AD, raising the prospect of occurrence of the disease significantly increases with age, which highlights the need to develop a new point of care, diagnosis, and prevention methodologies.[14] Lamentably, the biggest issue associated with AD therapy is that the late diagnosis of AD. Concerning this, specific and reliable approaches to avoid or delay the progression of AD are urgently needed.[1] In this relation, multiplexed devices can be built to simultaneously check several AD biomarkers for a speedy, inexpensive, and precise diagnosis and that helps to improve the understanding of a biomarker, molecular processes, and events that emphasize the pathophysiology of AD.[15] While numerous diagnosis techniques are available, we still face certain challenging factors that significantly obstruct their overall applicability.[1] In this shade of light, the more efficient AD monitoring strategies may allocate for earlier sensitive and selective diagnosis. Therefore, early detection of premature symptoms (such as the presence of biomarkers) of AD development before medical history may be crucial for the progression inhibiting/slowing of the AD. In our point of view, this early detection is the lack of an important factor, which could dramatically delay or even reverse the AD progress. In the past decade, the number of studies on developing fast and usable platforms for analytical detection using biosensor has increased substantially, since these devices have various advantages viz., high selectivity, sensitivity without having to sample large quantities.[16] Today's biomedical diagnostic sensors (biosensors) are widespread in different forms plus in the wide range of areas viz., monitoring of disease progression, patient tracking, environmental protection, food safety, pharmaceutical discovery, and biomedical science.[17] IUPAC describes a biosensor as a built-in transducer-receptor system, combining a biological identification item with a transducer, which converts the biological response into the quantitative/semi-quantitative analysis.[18] In the modern era of biosensing, SPR biosensor has emerged a potential for biosensing of several life threatening attacks of diseases/disorders. In the last decades, it has been extensively applied for *in vitro* diagnosis of several diseases/disorders.[19] Briefly, SPR has played a predominant role since its inception in the meadow of optical sensors.[20] It offers reliable benefits for the study of molecular (biological/chemical) binding events, especially, socio-economic, simplistic, real-time, label free, *in situ* method for recognition target.[21] Despite of this noteworthy merits of SPR biosensor, some challenging tasks including sensitivity, selectivity are there for researchers.[19,22] Therefore, this is a turn to investigate the pioneering approaches/strategies for highly sensitive and

selective recognition of target analytes. It could furnish the foremost milestone to biomedical sciences in terms of high sensitivity and selectivity.[1,19] Regarding this, for engineering high sensitive SPR biosensor, broad varieties of techniques have been preferred. Various types of pristine/modified (plasmonic and non-plasmonic) nanomaterials,[16] affinity/catalytic bioreceptors (antibodies, aptamers, enzymes, cells, deoxyribonucleic acid DNA) have been employed for highly sensitive plus selective detection of the established biomarker.[17] We witnessed that, in the arena of biosensing, several kinds of bioreceptors and nanomaterials along with or without modification/functionalization have been used. It may due to their tunable and versatile properties including selectivity, sensitivity, etc. in contrast to other engaged methods. Recently, the bioconjugates (bioreceptor and nanomaterial composite) is an attention-grabbing arena for the researcher scholars working on SPR biosensors for *in vitro* diagnosis of established biomarkers.[22] Literature mentioned that the flourishing employment of bioconjugates in SPR biosensor development provides exceptional merits in terms of selectivity, sensitivity, and reproducibility than the bare bioreceptors.[19,22,23] This confirms the importance of bioconjugates in the development of high sensitive and selective SPR biosensor. Besides, based on the severity and prevalence of AD, the different kinds of bioconjugates with numerous advances have been reported by researchers. We observe that the bioreceptors and bioconjugates have been efficiently utilized for detection of AD biomarkers that showed the remarkable outcomes in terms of AD biomarkers sensitivity and selectivity. Unfortunately, despite the indisputable benefits and advancement, until date less coverage has been given to bioconjugates. Therefore, there is a need to focus importance and resolve all issues related to the bioconjugates applications in the SPR biosensor for *in vitro* biosensing of AD.

To the best of our knowledge, this will be the review in the public domain, which bestows the insight of bioconjugates and bioreceptor-based SPR biosensors for highly sensitive and selective *in vitro* AD biosensing. This review provides insight on assorted important biomarkers of AD. It further addressed the various bioreceptors that facilitated the sensitivity and selectivity enhancement for SPR biosensor. The technical development in the domain of AD diagnosis has been discussed in brief. This review article further sheds light on the utmost unique properties of bioconjugates, which hold the interest of researchers for AD biomarkers and have contributed to the development of simple, rapid, and economic sensitivity enhancement methods. The present review article portrays insights concerning the adaptable idea of bioconjugates as an AD diagnosis platform using SPR biosensor. The article gives detailed insight into the analytical performance of bioconjugate-based SPR biosensor assay and their revolutionary benefits in terms of simplicity, accuracy, selectivity, and sensitivity for *in vitro* diagnosis of AD.

AD biomarkers

Currently, only an after postmortem histopathological evaluation of the brain and evident diagnosis is used to establish a confirmed diagnosis strategy for AD.[1,5] We witness, from its inception to date, medical records, laboratory testing, physical examination, neuroimaging, etc. are used for the clinical diagnosis of AD.[24] Besides this, the abundant literature estimated that the neurodegeneration of AD is starting 20–30 years before the clinical sign appears. Therefore, at early-stage diagnosis, therapeutic approaches can be more successful in AD therapy.[25] In this view, the early stage reorganization of appropriate AD biomarkers is therefore a more important footstep to differentiate the types of dementia and AD.[26] In this shade of AD diagnosis, we have enlisted several important AD biomarkers that playing a crucial role in the invention and designing of different ultramodern techniques to the AD diagnosis.

Biomarkers in cerebrospinal fluid

The precise diagnosis of neurological disorders by cerebrospinal fluid (CSF) biomarkers have been studied because they are trustworthy brain neurochemical measures.[27] As we know, the CSF is the most important source of AD biomarkers since CSF has a direct interaction with the brain and CSF composition may reflect the minute or large biochemical modification in the brain.[28] It has been reported that the CSF biomarkers viz. beta-amyloid$_{1-42/40}$ (Aβ_{1-42} and Aβ_{1-40}), total Tau protein (T-Tau, 6-isoforms), and phosphorylated Tau proteins (p-Tau-181) are important for both early detection of AD and development of advanced AD diagnostic strategies.[29] As well, it is worth mentioning that the above mentioned CSF biomarkers exhibited high (85%–90%) detection accuracy and good specificity.[29,30] Over several trials, the CSF test of AD suffering patients showed elevated p-Tau and t-Tau levels and a substantial decrease for Aβ_{1-42} in comparison to normal and healthy brains.[31,32] Despite the development and precision of diagnosis methods, to obtain AD biomarkers in CSF samples are the invasive techniques required to carry out a lumbar puncture that may lead to a serious headache in elderly patients.[33] Furthermore, the most preferred techniques for CSF containing AD biomarkers analysis i.e., enzyme-linked immunosorbent assay (ELISA) also suffering from several major demerits such as expensive reagents (labeling agents), slow and laborious procedure as compared to other advanced techniques.[34] There is an urge to develop the simplistic, rapid, socioeconomic, sensitive, and selective technique for the recognition of CSF biomarkers. Recently, carbon-fiber microelectrodes,[35] two-photon Rayleigh scattering,[36] etc., have been successfully applied in the AD biomarker detection that to overcome the crisis of conventionally used methods. In the future, the minimal/noninvasive biosensing technique can establish a new era for the recognition of AD biomarkers in other minimally/noninvasive body fluids.

Genetic biomarkers

Literature mentioned that the genome/genetic mutation and polymorphism have been found in AD patients, which

demonstrates the connection between disease progression and predisposition.[37] As per literature, AD associate mutations are habitually inherited. Consequently, there is a major possibility of the development of AD. In this particular respect, single-gene mutations on chromosomes no 1, 14, and 21 cause malformations on presenilin-1 (PESN-1), presenilin-2 (*PSEN-2*), and amyloid protein precursor (*APP*), respectively.[27,38] Whereas, the remaining molecules are associated with APP cleavage and further accelerated the Aβ plaque generations.[27] It is noticeable that the ApoE gene polymorphism is the most frequently observed risk factor in AD. It has been reported that the ApoE containing the ε4 allele develops the risk of AD by reducing amyloid clearance that accelerates the sensible plaques generation rate.[39,40] Besides this, homologous genes, namely PSEN-1 and PSEN-2 encode the main proteins for a γ-secretase complex that is mainly responsible for the cleavage of APP.[27] Additionally, the mutation of these above-mentioned two genes resulted in the enhancement of the overall ratio of $A\beta_{1-42}$ to $A\beta_{1-40}$, which activates the neurodegeneration. Literature reported that a PSEN-1 mutation is a severe form of AD and is commonly observed in the above 25 age group peoples.[41] Unfortunately, to date, there is a single biosensor available for the recognition of these two genes and therefore, there is a major necessity to develop an advanced biosensor for the detection of the PSEN-1 and PSEN-2 gene. Regarding precise biosensor, the AD biomolecular structure is the most important part of the assortment of appropriate bioreceptor and approaches (bioassay) for high selectivity plus sensitivity. Nevertheless, lack of insight of the AD (pathology and heterogeneity) and many other important obstacles that impede the applications and particularly during the early stage of diagnosis of the above mentioned AD biomarkers in clinical practice. Therefore, there is a colossal necessitate for further concentration on the development of appropriate strategies using advanced materials and conjugations for highly sensitive and selective biosensing of AD biomarkers. The advent of nano-material based biosensor technology is highly advantageous and has removed the old problems of the traditional biosensor by modifying the bio-composites.[16]

Imaging AD biomarkers

Structural imaging biomarkers

Structural magnetic resonance imaging (MRI) is one of the simplest, inexpensive, and increasingly applied neuroimaging techniques for AD diagnosis. The widely used T1 series demonstrates a strong contrast between gray matter and white matter.[42] Therefore, brain structural MRI results are recognized as a marker that could be identified in the later stages of neurodegeneration diseases.[43] Literature mentioned that AD diagnosis will usually experience hippocampal atrophy in contrast to cognitively intact older adults (Sn = 95%; Sp = 92%) in addition to entorhinal cortex atrophy (Sn = 90% and Sp = 94%).[43,44] On the account of the distinction between AD and non-AD dementia, sensitivity, and specificity values decrease to 79% and 81%, respectively.[45] As per the published data from a study by 50 patients with mild cognitive impairment of which 20 patients converted to AD within the next 2 years only. AD neuroimaging initiative showed that atrophy of the temporal lobe on the MRI used as a single biomarker had the maximum assessment precision (about 72%) followed by a positron emission tomography (PET) with the use of Pittsburg B (PiB) compound of the lateral temporal cortex and an MRI of the entorhinal cortex and hippocampus (68%).[43,46] The findings were clarified by the diagnosis of late mild cognitive impairment in patients in this case, which affected their cognitive testing more and did not reflect the full range of mild cognitive impairment spectrum.[43,47]

Functional imaging biomarkers

Abundant literature studies showed another functional imaging marker of neuronal injury is 18F-fluorodeoxyglucose-PET (FDG-PET).[48] As per literature, patients with AD or amnestic moderate cognitive impairments, who later become AD (in temporoparietal areas) have decreased glucose consumption. Additionally, the posterior cingulate cortex represents neurodegeneration in these regions.[49] Amusingly, FDG-PET also indicates the neuronal activity through the determination of the glucose metabolic rate in the brain, which is normally compromised before structural anatomical brain changes are recorded. It also illustrates vascular defects and blood-brain barrier disorders often observed in AD patients.[48] The sensitivity and specificity to distinguish AD from healthy (elderly) controls are seen in the FDG-PET (>90% for both parameters). Furthermore, the specificity of AD and other forms of dementia (including mild cognitive impairment) for discrimination is down to 78%.[45] For the mild cognitive impairment, FDG-PET is a small improved performance correlated with structural MRI for determining the transition to AD with higher sensitivity (88.8%) and specificity (84.9%) for FDG-PET, respectively compared with MRI of brain sensitivity (72.8%) and specificity (81%).[50] Over the last decade, the FDA has produced and approved multiple amyloid tracers with different half-lives and Aβ plaques affinities to envisage *in vivo* amyloid deposits as a research diagnostic method for AD pathology.[43] The Pittsburg Compound B (PiB) was developed for the first time. Its limits include a short half lifetime (about 20 minutes) and on-site cyclotron specifications and $_{11}C$ radiochemical knowledge and expertise needed.[51] Interestingly, PET scan using PiB showed high sensitivity (80%–100%), but low specificity (46%–88%) for the identification of mild cognitive impairment patients who would be converted to AD in a Cochrane meta-analysis of 9 studies.[52] New fluorinated tracers namely florbetapir, flutemetamol, and florbetaben are increasingly used with longer half-lives that display clear association with PiB and amyloid brain deposition on autopsy.[53] Florbetapir demonstrated low sensitivity (67%) and specificities (71%) in patients with mild cognitive impairment who progressed to AD in four years. In people who proceeded to AD over a span of two years, the PET scan employing this ligand displayed a sensitivity of 89% with an additionally lower specificity of 58% that restricting routine use so that the transition into AD is

predicted accurately.[54] Interestingly, a florbetaben analysis demonstrated strong levels of sensitivity (100%) and specificity (81percent) to forecast progression to AD.[55] Flutemetamol has also been shown towering ability in a further attempt to detect progression from mild cognitive impairment to AD dementia during 2 years of follow-up with excellent sensitivity (89%) and an appropriate specificity (about 80%).[56] Furthermore, CSF containing $A\beta_{42}$ ($p < 0.0001$) and p-Tau ($p < 0.006$) levels were closely associated with F-flutemetamol PET in a trial involving 34 patients who were diagnosed clinically with AD.[56] Unfortunately, no evidence has been found to support any biomarkers supremacy over the other (CSF versus imaging) in diagnosing AD. The preference of biomarkers relies in large part on the price and accessibility. However, some people are advocating the supremacy of imaging over fluid biomarkers because photography can differentiate both temporally and anatomically the multiple phases of the disease.[43] As per the literature, contrary to the amyloid imaging strategy, CSF pathology tests for $A\beta$ or Tau proteins have not achieved regulatory authorization for inclusion in clinical trials as a diagnostic method. Whereas the CSF biomarkers did not differentiate between AD and other types of dementia despite high sensitivity and unique characteristics of AD dementia from cognitively intact checks.[57]

Biomarkers in blood

Primary biomarkers are required to fulfill the requirements of the world's ever-rising aging populations. Plentiful literature mentioned that the invasion of CSF affects early detection, disease control, or the efficacy of drug therapy.[58] Besides this, only in specialist centers, PET imaging is available and it is a very precious technique. Recently, scientists have attempted to diagnose neurodegenerative diseases/disorders based on the molecular level changes in blood or cellular samples of patients. AD must be detected employing body fluids, including blood (serum, plasma), that are minimally invasive and even not invasive biomarkers. Owing to this, various omics approaches viz. metabolomics, proteomics, lipidomics, etc. have been reported for the recognition of blood-based AD biomarkers.[59] While more confirmation is needed for some of the blood containing AD biomarkers, it seems as reliable as CSF containing and genetic AD biomarkers.[60] Currently, AD's suspected blood biomarkers include proteins associated with $A\beta$, Tau-related proteins, etc. Besides, important factors involved in AD viz. brain aging, cell death, neuroinflammation, and cerebrovascular dysfunctions contain several blood biomarkers viz., plasma melatonin, cortisol, homocysteine, and prolactin levels.[60,61] That several recent findings have already demonstrated that the persistence of AD correlates with biomolecules for example serine, creatine, glutamate, phospholipids, myo-inositol, blood dehydroepiandrostone, 5-hydroxycytosine, N-acetilaspartan, etc. present in the blood and other biofluids excluding CSF.[62] Additionally, evidence from current research suggested that certain, blood (plasma) protein levels viz., interleukin 17, apolipoprotein A1, clusterin, pancreatic polypeptide Y, alfa 2-macroglobulin, etc.) with amyloid brain burden have been linked.[63] Recent information has found possible blood-based AD biomarkers viz., neuronally derived exosomes levels of $A\beta_{1-42}$, p-Tau, neurogranin, etc., which anticipate the risks of AD and risk of AD progression and differentiate between disease and cognitive ordinary older adults.[39,60,64,65] Unfortunately, the brain-specific proteins represent the AD molecular mechanistic insight at far lower concentration levels in the AD patient blood than the CSF sample, which must be quantified (excluding from the requirements concerning the analytical specificity) in other proteins (e.g., albumin and immunoglobulins). In this regard, few ultrasensitive methods (for example single molecule array) are available for the detailed analysis of AD biomarkers [i.e., Tau protein, neurofilament light chain (NFL) protein] present in blood samples.[66,67] Fascinatingly, appropriate combinations of blood-based AD biomarkers, cognitive testing, brain imaging, and clinical data are expected to bestow a more complex AD diagnosis/ AD therapy response than separate biomarkers. Literature reported that the NFL is a biomarker of large-caliber myelinated axons damages that indicate a strong link between CSF and blood. In this context, a commercial assay on a CSF and the same anti-NFL antibody pair has been performed. Calibration on the revolutionary Simoa assay for blood samples (including plasma/serum) has been performed using blood calibrator (plasma or serum) on the Simoa blood platform.[68,69] The first Simoa technique for quantifying the axonal protein NFL in clinical blood samples was reported. In this case, the Simoa techniques offer superior sensitivity than electrochemiluminescence, standard ELISA, and other techniques (using the same anti-NFL antibodies). It means, the NFL can be able to distinguish the AD from normal individuals by measuring the NFL plasma levels.[69] The latest AD centered neuroimaging initiative cohort research has shown a marked rise in the plasma NFL in AD cases (149% of control levels) with a receiver-operating characteristic under a curve value of 0.87, which can be equated with an important AD CSF biomarker. Although, a change in the mild cognitive impairment community was lower, plasma NFL was the highest in mild cognitive impairment cases with positive amyloid PET scans and expected faster cognitive decline, higher potential rates of brain atrophy in the future measured by MRI.[66] A key piece of information is that NFL high plasma is not an AD-specific feature. In certain neurodegenerative diseases, such as frontotemporal dementia, corticobasal syndrome, etc. increased levels were observed. Potential future use for plasma NFL is to assess patients with cognitive disabilities, for example, in the primary care unit, for the first clinical assessment. Concisely, plasma NFL could be an easy, noninvasive, and cheap tool to screen mainly for excluding neurodegeneration.[70]

Plasma biomarkers

Experts have attempted to categorize the cellular and molecular changes in AD conditions. It is incredibly important to detect the AD biomarkers through the use of

minimally invasive and even noninvasive fluids such as serum or plasma.[59] On the account of accurate detection of AD, several researchers paying attention to plasma biomarkers by samples collected using a simplistic noninvasive method.[71] In this way, two varieties of important potential AD biomarkers have been reported after a successful screening of AD patient plasma that includes common biomarkers such as fetuin B, Clu, pancreatic prohormone, etc. The next category includes especially AD-associated biomarkers such as Aβ, Tau protein, and apoE.[24,71] Generally, these biomarkers can be used for a variety of ailments diagnosis that is solely based on the blood level of biomarkers. Overall, several studies have confirmed that the above-mentioned biomarkers extremely different in each stage of the disease.[27] The plentiful data reported that the inability to reproduce the result is primarily because the sample selection methods and the quantification of these biomarkers were not methodologically consistent.[59,72] Several plasma AD biomarkers look as specific as CSF-based and genetic AD biomarkers but further testing is needed.[59]

Biomarkers in saliva

There is no doubt that for population screening a blood biomarker is much more achievable than PET or CSF, but it faces still some logistics limitations.[73] Saliva has been suggested as a possible source of biomarkers that can be easily obtained to diagnose and assess the risk of pathological conditions not only in the mouth but also systemically.[74] In response to the cholinergic innervations of cranial nerves VII and VII, the main salivary glands secrete saliva which the autonomous nervous system monitors.[75] This correlation with the nervous system implies that these secretions of the saliva from the gland may reflect different neuronal physiologies. Furthermore, plenteous literature revealed that the proteins of the central nervous system are secreted into the saliva through an age-dependent manner.[73] Besides, active transportation, passive diffusion, or microfiltration can transmit blood proteins into your saliva.[76] For these purposes, saliva may contain novel CNS injury biomarkers or maybe an additional and more usable source of AD-related biomarkers currently being used with eager interest in the blood.[73] It remains uncertain which source excretes the biomarkers into the saliva.[77] Currently, the available data demonstrate that the Aβ_{1-42}, A$\beta_{1-40,}$ and multiple Tau proteins present in saliva which is confirmed using conventional immunoassays.[73] A literature survey proved that the additional candidates for potential salivary biomarkers in AD are lactoferrin, alpha-synuclein, acetylcholine, etc., which seems valuable.[73,77] Many experiments are required to determine the diagnostic accuracy of these markers, using larger samples and standardized collection, processing, and analytical methods.[43] Besides, the assessment of the levels of biomarkers should include poor oral hygiene and the impact of slowing saliva flows in AD patients.[43,77]

Overall, this section provides a point-by-point audit about several important AD biomarkers classification, which is generally preferred for the diagnosis of AD through different modified biosensors. From a future point of view, there is a demand to develop ultramodern techniques using revolutionary nanomaterials with a combination of suitable biotransducers. In this contest, the bioconjugate and bioreceptor-based nanoarchitectured high sensitivity plus selectivity SPR biosensor have been designed which also provides the potential for early and accurate diagnosis of AD.

Technical developments for AD diagnosis

From its inception, the different techniques viz. mass spectrometry (MS), MRI, PET, ELISA, immunohistochemistry (IHC), flexible Multi-Analyte Profiling (xMAP), Western-blot, etc have been used for diagnosis of AD. In this subsection, the recent advances in the domain of *in vitro* diagnosis of AD have been reported. A literature survey reported that the despite advantages above mentioned AD diagnosis techniques are suffering from some major limitations, which restrict their application in the detection of AD biomarkers and accordingly AD diagnosis.[33] Additionally, many clinical results also claimed that the SPR offers excellent agreement in the detection of biomarkers than the ELISA assay, chemiluminescence, liquid chromatography-MS (LC-MS), Polymerase chain reaction (PCR), etc.[78] The limitations of engaged techniques have been tabulated in Table 1.

Novel mass spectroscopy

From its inception, MS is a well-known means in biological fluids such as blood and other biofluids for analysis of different AD biomarkers.[79,80] The high selectivity is the principal strength of MS and is therefore favored even as a reference tool for immunoassays. MS has previously been applied to employ a targeted MS (multiple reaction monitoring or selected reaction monitoring) or objective unbiased approaches (viz. proteomics, metabolomics) to blood-biomarker discovery and validation in AD. A literature survey reported that, compared to CSF, biomarker tests in the blood have multiple advantages, including more convenient for patients, less expensive, and more suitable in longitudinal evaluations for repeated sample selection. Regarding this, MS offers the potential to detect AD biomarkers in blood with remarkable sensitivity and selectivity.[79] In MS, usually, the mass-to-charge ratio (m/z) is used to measure the target analyte. Recently, hybrid MS has been reported for the detection of AD biomarkers. Fascinatingly, more than one mass analyzer is used in current hybrid MS instruments. This method is known as multiple reaction monitoring/selected reaction monitoring or parallel reaction monitoring according to the mass analyzer used. Different AD biomarkers viz. Aβ, Tau protein, apoE, phospholipids/sphingolipids, acylcarnitines/fatty acids/triglycerides, cholesterol and related metabolites, etc. have been detected using novel hybrid MS.[79] Present measurement efforts for the main AD biomarkers namely Aβ_{42}, T-Tau, and p-Tau battle the high complexity of this matrix and low protein abundance in immunoassay blood. In combination with immune methods, MS techniques can be used to overcome this issue,

Table 1. Reported AD diagnosis techniques and their disadvantages.

S. No.	Techniques	Disadvantages
1.	MRI	1. It is a costly operation. 2. It is an insensitive method of calcifications. 3. It has a poor scan speed. 4. Motion artifacts
2.	PET	1. It is an expensive process. 2. It has a poor spatial resolution. 3. Artifacts of movements
3.	AD-associated proteins (ADAPs),	1. It is a costly process. 2. It required strict low-pressure. 3. It result may alter e with energy, pressure, collision gas, etc.
4.	ELISA	1. It is time consuming and inefficient process. 2. It is insensitive to the lowest concentration level biomarkers. 3. There are major chances of false positives.
5.	xMAP	1. It restricts the use due to the cost factor. 2. It provides low to medium resolution results. 3. It contains a small number of heterozygous ambiguities.
6.	Western-blot assay	1. It contains lower stability. 2. An inconsistency in every step can skew the whole process.

which Nakamura and his colleagues have shown successfully in the treatment of Aβ peptides.[79,81] Recent innovations in MS-based proteomics provide important clinical tools in biomedical fields. Recently, proteomics MS is gaining much attention from researchers for AD biomarkers detection in CSF and blood samples. A crucial purpose of proteomics MS is the identification and quantification of biological sample proteins. Proteomics was focused on MS.[82] In recent attempts, Thomas McAvoy and his colleagues have established the quantitative detection of Tau protein in CSF samples based on highly sensitive and selective pioneered technology i.e., immunoaffinity LC-tandem MS technology. The technology has applied a monoclonal antibody (mAb) to enhance Tau protein selectively in CSF and tryptic digestion to create proteotypic peptide fragments.[83] Despite the indisputable benefits of MS, it suffers some limitations such as it is a much expensive technique, it requires strict low pressure and it may vary with energy, pressure, collision gas, and other factors.[33]

Ultrasensitive immunoassay for AD diagnosis

In this context, Yang and others have been created a sandwich ELISA assay for the measurement of low concentrations of natural Aβ oligomers in human brain samples. In this study, the immunoprecipitation of Aβ in provided CSF was performed with specific antibodies and the immunodepleted solutions were transferred to new tubes for further study. They also removed some negative outcomes by spiking experiments, due to molecules in CSF, which interfere with the immunoassay. Therefore, these tests were a helpful and sensitive tool for the quantification of Aβ oligomers in several biological samples.[84] ELISA or xMAP testing to measure AD containing CSF biomarkers (viz., $A\beta_{1-42}$, T-Tau, and p-Tau 181) is defined by Kang et al. Using Luminex xMAP technology, a multi-parameter perforated prototype bead assay for simultaneous measurement of $A\beta_{1-42}$, T-Tau, and p-Tau181 in CSF samples was developed.[85] Despite the indisputable merits of ELISA, it suffers several demerits including 1) it is a time-consuming and inefficient process, 2) it is an insensitive method to low-level biomarkers, and 3) also there are major chances of false positives.[33] In 2020, for measuring all CSF biomarkers ($A\beta_{42}$, T-Tau, and p-Tau 181), a completely automated system is based on the chemiluminescence enzyme immunoassay (CLEIA) was implemented. Interestingly, this CLEIA assay offers several noticeable merits, including reliability, ease to perform, rapid, cost-effective method, etc. than the ELISA. The authors claimed that the CLISA provides good diagnostic accuracy for all AD containing CSF biomarkers.[86] Recently, digital ELISA known as a Single-molecule array (Simoa for diagnosis of neurodegenerative disorders/disease) has been developed and commercialized by Quanterix.[87] In another pioneering work, the sandwich enzyme-linked immunoassay (EIA) has been described for the detection and measurement of AD-associated proteins (ADAPs), which confirmed that it is a unique biochemical assay that can be used for the diagnosis of AD after postmortem.[88] The West-blot detection test could skew the entire process due to imbalance at any stage of the procedure and it also suffers the stability issue (low stability).[33]

Biosensor: surface plasmon resonance

The biosensor has been established over the past 50 years and in the last 10 years, working in this area has contributed terrifically to academia. Such speedy evolution has been primarily owing to the advancement of miniaturization and the admirable advancement in the fabrication methods in the arena of biosensors across recent decades. Additionally, the utilization of novel molecules (bioreceptors) and ultramodern nanostructured nanomaterials for biorecognition has gained more attention from scientists.[17] Generally, the biosensor is an analytical device (Figure 2) that unites a biological component with a physicochemical detector consists of biotransducers/bioreceptor, a transducer, and a detector (i.e., digital output).[22] In a nutshell, the transducers identify the interest biomarkers (analytes) by reaction and convert the response into a quantifiable signal for molecular changes. More specifically, the target biomarkers interact with the engineered bioreceptor in the biosensor, which generates the response/signal to the

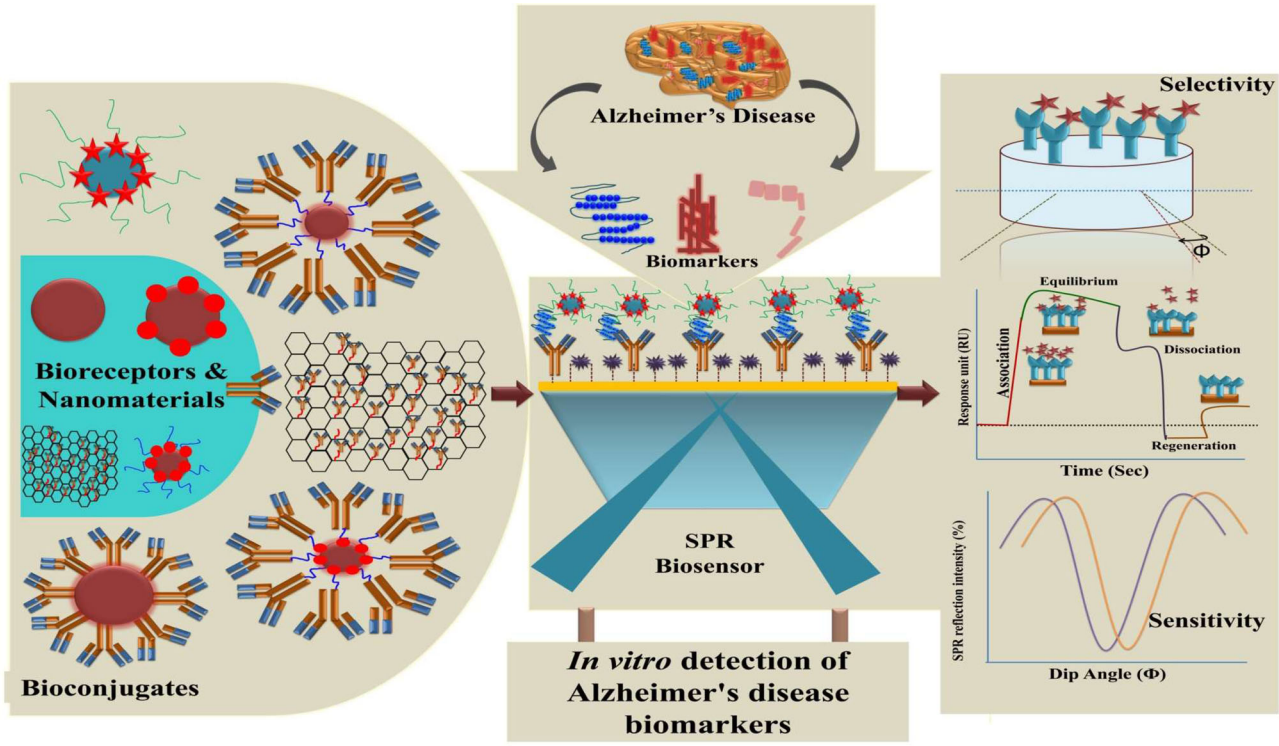

Figure 1. Nano-architectured bioconjugates and bioreceptors mediated SPR biosensor for highly sensitive and selective in vitro diagnosis of AD biomarkers.

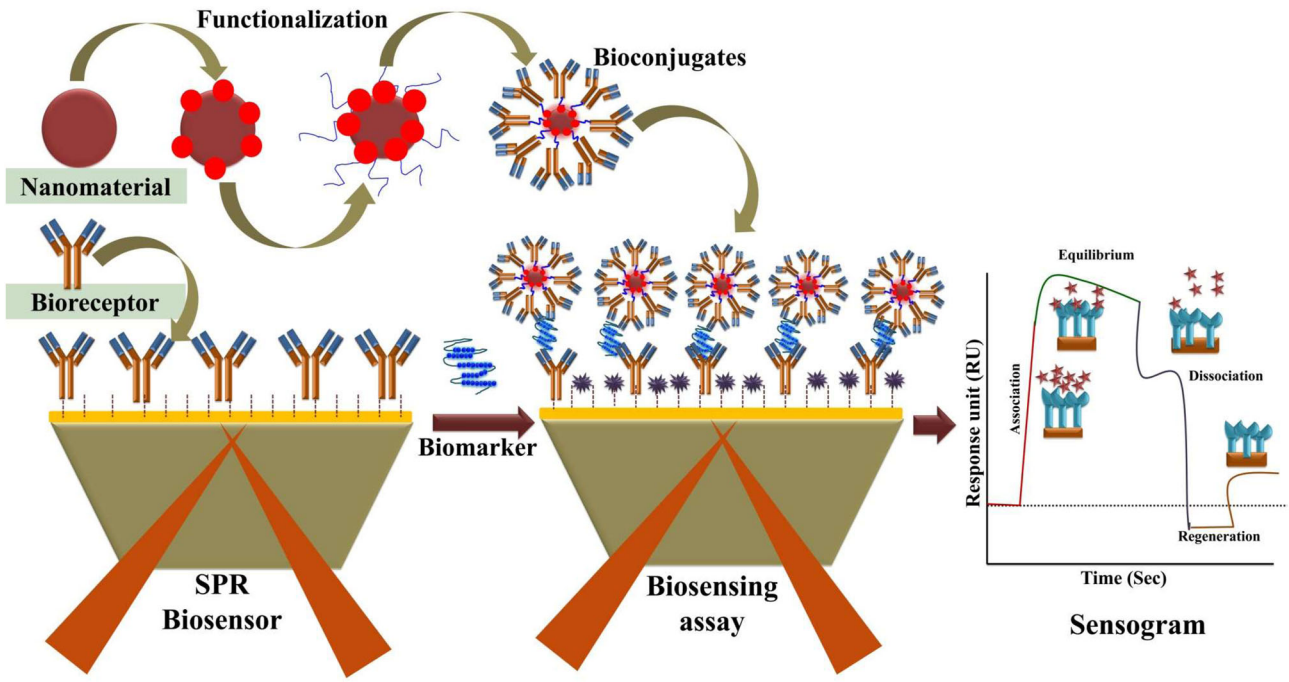

Figure 2. Ultramodern fabrication scheme for sensitivity and selectivity enhancement of SPR biosensor for in vitro diagnosis application.

transducer.[19] Besides, diverse types of bioreceptors (affinity, catalytic) are preferred viz., antibody, aptamers, enzymes, DNA, etc., depending on the interaction with the interest of biomarkers (Figure 1).[89] Optical biosensor seems to be the principally popular biosensor that includes an optical transducer system based bio-recognition feature. In that, the interaction between biomarkers and bioconjugates/bioreceptors generates an optical signal for qualitative and or quantitative measurement. In this regard, owing to the urgent requirement for the biomedical field, assorted types of biosensors viz. piezoelectric, electrochemical, optical (SPR), etc. have been fabricated to date.[22,89] Many SPR based biosensing methods for the identification of biomarkers have been used in recent decades (Figure 2). This SPR based biosensor can detect molecular changes that can help to the monitoring the disease/disorder state and

ultimately patient health.[78] Many studies involving the detection of AD biomarkers using SPR biosensors have been published in various reputed journals.[33] In a short period, the SPR biosensor gives excellent results with higher sensitivity and selectivity. In addition to this, it provides a socioeconomic, simplistic process for *in vitro* diagnosis of AD biomarkers. In this way, it can be a wonderful substitute for the recognition of AD biomarkers than conventional methods.[33] In the last several years, technology has expanded exponentially in terms of increasing the numbers of advanced methodologies and numbers of publications, since the very first involvement by the Biacore group (GE Healthcare) in 1990.[19,90] Till July 2020, a total of 26,171 scientific papers are published in the public domain as per PubMed.[91] SPR has developed from a relatively impenetrable physical phenomenon to a commonly used optical instrument for the biological and chemical investigation to deals with the binding event among analyte and other molecules.[19] In SPR biosensor technology, the SPR phenomenon was found between the metal surface and medium (liquid/vacuum/air) with the analyte (specific biomarker). If the particular molecule unique to the receptor (site) of these elements identified, this consequently resulted in the change in the metal surface that creates an angle shift (Figure 2). Therefore, the alteration in the refractive index on the metal surface caused this shift in angle.[92] It means SPR biosensor can be used for the various *in vitro* diagnostic applications through immobilizing the bioreceptor (ligand) at the surface of the selected metal surface sensor chip. As a biomarker and bioreceptor interaction produces the estimable change of the refractive index close to the metal surface, which causes a change in the SPR sensorgram angle and consequently resulted in a detectable response.[93] In the brightness of the tunable properties of SPR biosensor, the (coated) surface of the sensor chip can be immobilized by a range of bioreceptors (biomolecules) using numerous varieties of chemistries with a coupling of carboxyl, thiol, amino, hydroxyl, and aldehyde functional groups.[19] One well-known approach is to combine bioreceptors chemically on the surface of the sensor chip and then capture its specific biomarker. Briefly, the bioreceptor-captured analyte in turn can be utilized to sense any of its precise binding partners (antigen).[94,95] In recently published work, the potential of biotin, glutathione-s-transferase, and histidine-tagged fusion proteins have been efficiently used in the SPR biosensor development for sensor chip surface modification and capturing the bioreceptors.[19,94] With several benefits, SPR biosensor can be extended to an assorted variety of applications without the fluorescent and radiological markers (labeling agents) meant for a broad spectrum of interest of analytes.[94] Further, the SPR provides numerous advantages such as details about binding kinetics, real-time, rapid, and continuous measurement, required small amount and volume of samples, sensitivity, and specific binding events, and multiple-use (generic method). Additionally, the SPR biosensor determines the interest analyte concentration in the absence of a standard.[19,94,95] Within the low concentration ranges, SPR sensing can detect analytes with a picomolar to nanomolar (pM to nM) limit of detection. Besides, SPR biosensor will perform research in a wide variety of biofluids such as whole blood, plasma, urine, saliva, etc. in complex matrices, and obtain much of the possible clinical samples by emphasizing the flexibility of these sensory platforms.[96] Apart from such exceptional merits, the conventional SPR biosensor is not so sensitive and selective to the interest analyte, especially smaller biomarkers, which is a major constraint of SPR biosensor and accordingly, it retains the biological potential of SPR.[19,22] Therefore, the key challenge for biosensing using SPR biosensor is to ensure that the biomarker recognition in the early stage of disease in a true sample is highly sensitive and selective. In this way, one of the major challenges for biosensing is to build SPR biosensors that are highly selective and sensitive by improving their surface area, optical properties or conductivity, specificities and electrochemical behavior.

Bioreceptors

A biosensor is primarily intended to provide analysis specificity for biomarker/analyte. Specificity demands a selective and strong connection between the biorecognition element and the precise target. There are several kinds of elements of biorecognition, which generate distinct structures to regulate the output of biosensors uniquely. Firstly, before a detailed study of biosensor performance can occur, it is first crucial to understand the particular features for every biorecognition element. The appropriate assortment of bioreceptors or biorecognition elements is paramount for the successful development of a biosensor such as an immunosensor. The various kinds of bioreceptors have existed for the fabrication of high sensitive and selective SPR biosensors such as affinity bioreceptors and catalytic bioreceptors. In the type of affinity bioreceptors, mainly antibody, antibody fragments, aptamers, deoxyribonucleic acid (DNA), peptides, molecularly imprinted polymers (MIPs) are widely utilized for the construction of bioconjugates followed by SPR biosensor.[97] On the other hand, the catalytic type of bioreceptors includes enzymes, organelles, microbes, tissues/cells, etc. Concisely, specific biomarker binding to the surface of the transducer is an approach with an affinity biosensor. The catalytic biosensor is another kind of biosensor that has led to a novel biochemical reaction.[98]

Antibody

The antibody is the glycoprotein that is formed by host specimen B-lymphocyte cells as a reaction to the existence of foreign material known as an antigen.[99] The antibody is often referred to as immunoglobulin. As per literature, there are five types of antibody, out them IgG is the most widely used in the manufacturing of immunosensors.[100] With IgG, its Y-shaped structure is made of two identical light chains (MW approximately 25,000 Da) and two identical heavy chains (MW approximately 50,000 Da), connected by sulfite bonds and hydrogen bonds (i.e., non-covalent interactions).[97,101] Generally, light and heavy chains have a

variable as well as constant domain. Briefly, one variable domain for both heavy chains (V_C) and light chains (V_L).[100] Besides, one constant domain for C_L along with three for heavy chains namely CH_1, CH_2, and CH_3. In this respect, the variable region i.e., V_L and V_H are always the most significant in the antigen and antibody reactions and differ among various antibodies, providing the specific binding properties based on the sequence of amino acids.[100,101] In this gloom of specific binding, there is the complementarily determining regions and present hypervariable loops representing the bonding site to the antigen. Furthermore, the high diversity of region-specific complementarily allows for the production of high-specific antibodies for many types of antigen. In the case of IgG, the antigen-binding fragment (Fab) and non-antigen binding fragment (Fc) can be separated into an IgG.[102,103] Additionally, the antibody is bivalent that can simply connect two antigens with the Fab region having two same antigen-binding positions (paratopic). Whereas, the antigen complementary site is called "epitopic," because it is based on size, form, and chemical compatibility.[101] Fascinatingly, antibodies interact with their antigens through numerous binding forces viz. ionic/hydrogen bonds, van der Waal forces, and hydrophobic interactions.[100,104] Nonetheless, for non-covalent interactions, a close fit determined by the high degree of complementarily within antibody and antigen is required.[105] In certain cases, the antibody-antigen binding episode comes with conformative changes in antigen, antibody/or both, that further introduce it closer together but it might also adversely impact the binding affinity.[106] Concerning antibody production, the immunization of the host (animal) can lead to antibodies, but these are thorny and time-consuming methods. In contrast, the recombinant antibody production is a faster process and enables a wide variety of antibodies to be made.[101,107] In the line of a monoclonal antibody, it can be produced from identical immune cells. The monoclonal antibody is typically admirable for a high affinity to a single epitope.[107] Also, it provides a high degree of specificity to the antigen and is majorly used as a primary antibody for sandwich assay or immunosensor development.[97,108] Despite the various antibody types, polyclonal antibodies are acquired as a combination of immunoglobulin for an antigen from numerous immune cells, which are each binding a specific epitope. This obtained polyclonal antibodies are generally used in immunosensor as secondary antibodies.[106,107] To optimize the high sensitivity of SPR biosensors, the maximum specific antibody binding to the antigen attached sensor surface is required. In comparison, antibodies have usually been used to build target-specific SPR biosensors for biomarker recognition.[109] An actual fundamental mechanism deals with the specific antigen and antibody interaction process. The selected antibodies are typically bound to the surface of the used transducers by covalent bonding like amide, thiols, or ester bonds. In this way, the surface of the selected transducer must be changed using polymers, monomers, or linkers. It initiates attachment to the antibody by amino, carboxy, sulfhydryl, and aldehyde groups, for the conjugation/immobilization of antibodies on the surface of transducers or nanomaterials.[107,110] To date, various forms of antibodies have been used clinically for specific immunosensors and in the industry. It should be pointed out that these types of sensors are more sensitive and quicker to screen in comparison to traditional assays.[97,107] Therefore, owing to the obvious merits of antibody, it has been broadly investigated in AD biosensing applications.

Aptamers

Aptamers have recently been receiving considerable attention in architecture, analysis, and diagnostics as biosensor recognition components.[97] In general, aptamers have characteristics comparable or even better than conventional detection elements, for example, antibodies.[97,101] As mentioned, antibodies have a limited association (affinity) with target molecules, resulting in lower responses, whereas aptamers could bind to hundreds of different sizes and structures. Aptamers are small nucleic acids or oligonucleotides viz. ssDNA or ssRNA related structurally to other molecules, so they may be strongly affinous, unique to their targets.[97] Interestingly, aptamers can selectively bind to the low molecular weight molecules, including dyes, amino acids, etc. Additionally, they also bind to the small parts of macromolecules viz. polypeptides, proteins, and polysaccharides.[111,112] During the development of aptasensor, effective aptamer selection is usually carried out for target molecules viz., proteins, enzymes, nucleotides, ions, etc. based on small and large targets. Whereas, hydrogen bonding, hydrophobic interactions, electrostatic interactions, etc. are generally involved in the binding of the aptamer to its target analyte.[97,101] The synthetic preparation provides the other admirable merits such as stability (heat, wide pH range, and salt concentration), a broad range of target analytes, repeatability, more resistance against denaturation, etc making aptamers ideal for reuse, chemically modifiable to improve its stability, affinity as well as specificity.[113] Therefore, owing to the noticeable merits of aptamers, it has been extensively explored in AD biosensing applications.

Miscellaneous

Miscellaneous bioreceptors including enzymes, nucleic acid, MIPs, etc.[97,114] have been widely utilized for the detection of interest biomarkers. Especially, enzymes gain bioanalytic specificity using hydrogen, electrostatic, and other noncovalent interactions to create patterns of identification through binding cavities buried within their 3D structures. In the case of the enzyme-based biosensor, it works based on biocatalysis, which means the enzymes have captured the target and converted it into a measurable product that can be frequently controlled using the transduction method.[98] Regarding a nucleic acid-based biosensor, the complementary binding motif of DNA is used to achieve bioanalytic species by nucleic acid biosensors, called genosensor.[115] In this context, once a DNA target sequence is identified, it can be designed and immobilized as a bioreceptors/biorecognition element in an artificial way on the sensor surface.

Furthermore, the precision of the immobilized DNA fragment and the target sequence is obtained by the special complementary detection pattern.[97] Concerning synthetic bioreceptors, MIPs are gaining much attention in biosensing. Generally, MIPs synthesized using a templated polymer matrix to obtain admirable analytical specificity via noncovalent bonding or electrostatic interaction or size-dependent patterns, etc.[116] Against natural bioreceptors, MIPs for each target analyte are can be synthesized.[97] Fascinatingly, the MIPs containing tunability solely relied on several parameters viz., the selection of functional monomer, solvent, cross-linker, and target or biomarkers, etc. In biosensors, MIPs are structured to embody the target biomarker, establishing synthetic patterns of reconnaissance among the biomarker and the polymer matrix.[117] There are other recognition elements beyond the ones mentioned in this review and alternatives can be explored before the biosensor is built. To summarize, we retain the basics of natural (antibody, nucleic acid, and enzyme), pseudo-natural (aptamer), and synthetic (MIP) bioreceptors for the context of this review, while we are auditing the *in vitro* diagnostic bioconjugate and bioreceptors based SPR biosensor for AD biomarker detection.

Nanomaterial in biosensing of AD biomarkers

Recently, new modified transducers have been produced based on the inimitable features of nanosized materials and the aptitude to customize their sizes plus structures. Generally, nanomaterials show remarkable optical-mechanical, electrical, and thermal properties because of the quantum size effects. They are considered among the most appealing ways of promoting the setup of biosensors with improved analysis.[118] A literature survey demonstrates that the incorporation of nanomaterials into SPR biosensors increases the overall conductivity plus the catalytic activity of the transducer.[19] Because of their high surface, it helps to immobilize a large number of biotransducer and improve the accessibility of special analysts.[119] Gold Nanoparticles (AuNPs), silver nanoparticles (AgNPs), carbon materials (e.g., multi-wall carbon nanotubes), silica nanoparticles and metal oxides (silver metal –tantalum-v-oxide nanoflakes), etc., all have unique basic functionalities to contribute to the latest transduction system, which is the most widely used nanomaterials in the production of the ultra-modern SPR based AD biomarker detections. Despite this, graphene and their derivative, magnetic nanoparticles (MNPs), quantum dots (QDs), etc are also efficiently employed in different types of biosensors but less extent for AD biomarkers detection.[16] Importantly, these nanomaterials have been documented for the production of biosensors that perform detection by a sandwich or direct detection.

Nanoparticles with different configurations and characteristics are the most commonly reported nanomaterials to build novel biosensors.[120] Due to their smaller dimension and large surface area, nanoparticles give singular physical, electronic, and chemical properties, which can be used as an amplifier to enhance overall signaling or to serve as a suitable platform in order to immobilize the biotransducer for the production of high-performance biosensors.[16,120] In the case of AuNPs, the AuNPs are amongst the more extensively used nanomaterials in the biosensors domain, owing to their astonishing optical and electronic characteristics, their regulated morphology plus size, and convenient preparation methods.[121,122] Furthermore, AuNPs exhibit a large surface area, high surface-to-volume ratio, good conducting capability, high catalytic activity, good chemical stability, ease of operation, biological compatibility, ease of operation, which help to immobilize bioreceptors.[16,23] Other nanoparticles, including AgNPs, MNP, and QDs were also used to build AD biosensors, although to a lesser degree.[16] Besides, AgNPs and their composites were investigated for developing biosensing platforms for the determination of several analytes with features viz., high conductivity, excellent biocompatibility, and enhanced electrochemical signal, etc.[123] The special features of MNPs (viz. metals, alloys, or oxides) included easy size control, low-cost processing, physical and chemical stability, good biocompatibility, and simple manipulation with magnetic fields, etc. opens the new domain of nanomaterials for AD biosensing.[16]

In particular, carbon nanomaterials viz., carbon nanotubes (CNT), fullerenes, carbon fiber, graphene, graphene quantum dots (GQDs), carbon dots (CDs), gained significant attention during the development of analytical biosensor instruments.[124] CNT and graphene are the most frequently used carbon nanomaterials for the fabrication of biosensors for AD biomarker detection.[16] While carbon nanomaterials differ in their specific characteristics, their significant characteristics are large surfaces, easy operation/functionalization, and towering surface-to-volume ratio, biocompatibility, electrical conductivity, electrochemical activity, and antifouling effect.[16,19,125] In the future, there is tremendous scope to use pristine graphene materials in the development of SPR biosensors and their applications in revealing disease/disorder biomarkers with high sensitivity plus selectivity.[19]

Currently, polymer-based bio-sensing technology is widely applied in AD biosensing,[16] because it is easily functionalized with bioreceptors, high sensitivity plus selectivity, hysteresis, and enduring stability. In the future, it can be an innovative substitute for currently engaged nanoparticles in SPR biosensor.[126] Overall, nanomaterials are widely used to boost the sensitivity with a selectivity of analytical procedures employing amplification agents that are typically updated with secondary recognition and labeling components.

The emerging era of bioconjugates in AD biosensing

Pertaining to the diagnosis of life-threatening ailments and for precocious disease/disorder diagnosis, quantitative and qualitative interest biomarkers recognition in body fluids is an essential footstep in clinical. Unfortunately, the complex and heterogeneous nature of clinical samples offers the most challenging task to the scientists for the recognition of the

interest of biomolecules along with high sensitivity plus selectivity.[127] As per literature, outstanding and majorly preferred diagnosis techniques are also suffering from several above-mentioned challenging problems. Recently, bioreceptors and bioconjugation is an interesting area of research that is expanding rapidly. In brief, for applications viz., disease diagnosis, ligand discovery, and high throughput testing, new methods have been developed through mild/site-specific modification of RNA, DNA, proteins, carbohydrates, antibody, antigens, etc. In this way, clinical chemistry takes advantage of the biomarker's innate properties to produce a good signal. Unfortunately, bioreceptor (antibody/aptamer/polymers) based on several clinical approaches are restricted to special cases because of various major limitations such as sensitivity and selectivity.[128] Therefore, SPR based biosensing has becomes favored for biomedical applications, and in a recent couple of decades, owing to previously mentioned major demerits, the efficient tuning of bioreceptors and nanomaterials (plasmonic and non-plasmonic) and their flourishing development has considered an exceptional substitute that can conquer the mentioned limitations of SPR biosensor.[22,109] Nonetheless, bioconjugates have become a part of the SPR biosensing network, encouraging the increase in selectivity and sensitivities representing the potential of many magnitude orders to the detection limits.[16,129] On the whole, biosensing required the production of effective and reliable methods to ensure that the resulting bioconjugates are stable, responsive, and reproductive.[19,23] In the shade of the above challenge list, various researcher groups have actively reported several numbers of methods along with advanced conjugation strategies for AD biosensing. In recent times, the paradigm improvements in the metallic crystals in a broad array of applications have given rise to outstanding and incredible properties, beginning from the voluminous to the micro-to-nano.[130] Fascinatingly, the integration and optimization of several types of nanomaterials into the biosensing system will lead to the production of highly responsive and precise point of care technologies.[16] Especially, the plasmonic nanoparticles from coinage metal have attracted great interest, thanks to their wide surface area to volume ratio, as transducers for the production of the optical biosensor (SPR).[16,33] Besides, the popularity of plasmonic nanoparticles stems mainly from the known localized SPR (L-SPR) which occurs when a light incident wave induced by conductors is trapped less than the lights of the incident light in conductive nanoparticles. The conduction band electrons in the nanoparticles oscillate along with the incident wave-induced resonance frequency.[131] Besides the era of plasmonic material, in the last decades, non-plasmonic materials are also taking a remarkable interest in the SPR biosensing to the diagnosis of AD.[16,19] The abundant literature revealed that the AuNPs, carbon-based material, including graphene and its derivatives such as graphene oxide (GO), reduced graphene oxide (rGO), etc. CNT, and several polymers, all have outstanding and venerable properties for developing a new SPR biosensor approach and are the most habitually used nanomaterial for developing SPR biosensor.[16,33] In addition to these nanomaterials, many admirable nanomaterials are often used, but not as much for example AgNPs, MNPs, QDs, and dendrimers. Attractively, such types of excellent nanomaterials have been recorded more frequently for the construction of SPR biosensor, especially biosensing basis on the sandwich or direct detection assay.[16,22,33] Taken as a whole, nanoparticles themselves are a superb tool in generating high contrast signals, but for most sensing, diagnosing they need to conjugate the functional molecule.[132,133] The incorporation of outstanding nanomaterial into the SPR biosensor has been showing the enhancement of the transducer conductivity, while at the same time it encourages the immobilization of an enormous amount of bioreceptors as a consequence of nanomaterials high surface area.[118,134] Indeed, to this, the functioning of appropriate nanomaterials has helped to build up a very selective and sensitive biosensor for AD biomarkers through integrating bioreceptor.[16,126] Further, there is a notable emphasis in the regards of immobilization of the bioreceptors on the surface of nanomaterials. It may due to an effect on the sensitivity, selectivity, and reproducibility in final outputs of the biosensor.[16,118,135] The literature survey divulged that the effectiveness of the SPR biosensor solely depends on its binding affinity and admirable specificity. On the other hand, the density of bioreceptor on the surface of the nanomaterials/transducers, and the orientation of bioreceptors are also important factors that affect the sensitivity and selectivity of SPR.[136,137]

Bioconjugates used in AD diagnosis

In this section of this review, we are recapitulating the several types of bioconjugates used in the AD biosensing via SPR biosensor (Figure 3). In the shadow of types of bioconjugates in AD biosensing, we look at the several significant strategies implemented for bioconjugation bioreceptors (antibody, aptamer, etc.) and nanomaterials [viz. AuNPs, AgNPs, multi-walled-CNTs (MWCNTs), etc.][19,27] In particular, the physisorption method or chemisorptions method can achieve by alteration of the suitable bioreceptor and nanomaterial. Due to the very versatile surface chemistries of nanomaterial, several bioconjugation strategies have been used to connect them with functional molecules. In that, the covalent bonds between amine groups in amide chemistry biomolecules have been commonly used to bind small molecules, proteins permanently on the surface of nanomaterials.[132] Moreover, the maleimide groups are often to use combine nano-material with the thiol group of other functional molecules. Based on chemical nature, this is especially suited for bioconjugation of the antibody with thiols outside the affinity side, so as not to disrupt binding to the interest biomarkers by connecting with nanomaterials.[138] Besides this, nanomaterial may also be coupled with several bioreceptors in an intermediate affinity group viz. biotin-streptavidin, electrostatic interactions with strongly loaded bioreceptors.[133] Lately, the techniques for the attachment of functional molecules precisely to the crystalline surface of the nanomaterial have gained much attention.[22] For

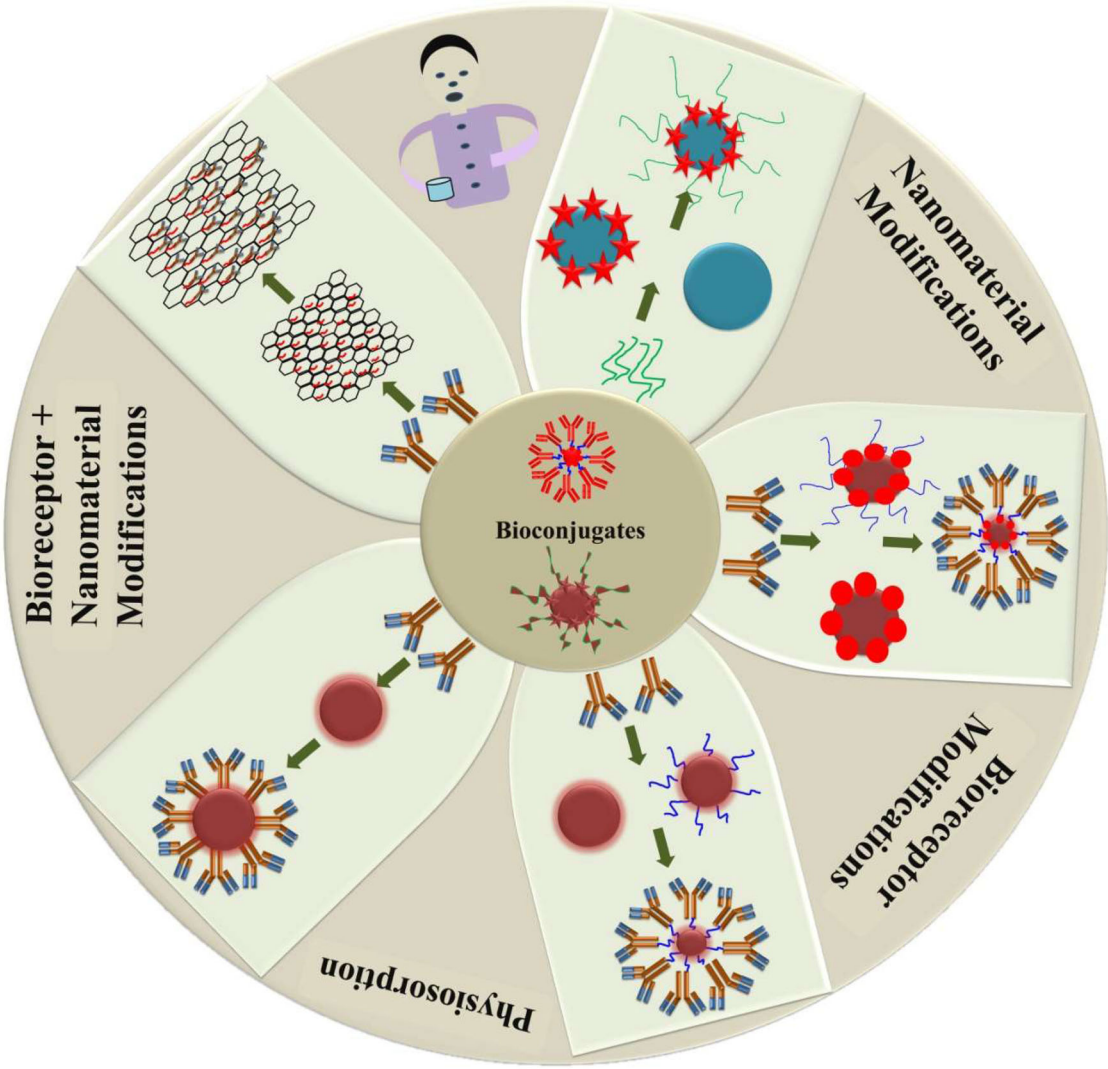

Figure 3. Bioconjugates used in SPR biosensor for *in vitro* diagnosis of AD through various bioconjugation methods.

example, owing to strong interaction with the surface atoms, thiol functional groups have been widely used in bioconjugation with AuNPs, and nowadays it leading several approaches for biosensing.[23] A key challenge in creating a very efficient biosensor is that many of the reported approaches require several chemical treatments that lead to the bioconjugations of bioreceptors in directions not ideal for binding analyts. This heterogeneity affects the performance of SPR biosensors. More research is still necessary for overall to develop a suitable technique to fully control bioreceptors on the surface of nanomaterial along with loading density and its orientations.[133] Choices of bioconjugates and conjugating methods have been developed to detect AD biomarkers with high sensitivity and selectivity. In a further report, we have discussed the numerous important types of bioconjugating methods.

Types of bioconjugation methods

In this segment, we discussed the various strategies for the nanomaterial-bioreceptor conjugation. Such policies are based on physisorption or chemisorptions by bioreceptor and/or nanomaterial modifications.

Physisorption of bioreceptors

Physisorption typically creates attractive forces between the nanomaterial (such as nanoparticles, carbon nanomaterials) and bioreceptors (such as an antibody, aptamers, MIPs, etc.), by electrostatic interaction, hydrogen bonding, hydrophobic bonding, and van der-Waals bonding.[101] Interestingly, physisorption is the simplest and quickest method of conjugation and it needs minimum surface chemistry expertise. Their ease is because neither bioreceptors nor nanomaterials have to be chemically modified. As we know that the bioconjugates with highly specific activity benefit integrally from the addition of a significant excess of bioreceptors to nanomaterial suspension. Unfortunately, the overloading of bioreceptors can cause a gradual loss of loosely organized bioreceptors. In this line, with each case considering bioconjugates stability and preservation of bioreceptor activity, an appropriate amount of bioreceptors should be preferred. In 2009, Lee et al. reported the AuNPs-antibody fragments bioconjugates using AuNPs and a

polyclonal antibody against $A\beta_{1-40}$, which was further stabilized using casein (0.1 mL of 5%) solution.[139] In general, for bioconjugates, many parameters such as the isoelectric points, pH, the addition of the bioreceptor quantity, etc. are considered during physisorption process preparation.[23] In this shade of light, this is widely acknowledged that when the pH is below or just slightly above its isoelectric point, optimum protein adsorption is achieved. In addition to this, bioconjugation is examined to determine the optimal value at several pH ranges in conjugation operation.[140] Eventually, various procedures provide a further step toward the stabilization of pioneered bioconjugates by the addition of bovine serum albumin, polyethylene glycol (PEG), etc. Such blockers, often protect any remaining free sites on nanomaterials and thus avoid further nonspecific contact and consequently offered the precision detection of interest biomarkers.[101] Given its simplicity, the physisorption of bioreceptors to nanomaterial offers few drawbacks viz. randomly immobilization of bioreceptors on nanomaterial and weak interaction forces. In the case of antibody, randomly immobilization can lead to loss of antigen-binding ability owing to the steric hindrance or direct interaction among nanomaterial and antibody.[23,101] In the conjugate sample, the AuNPs-bound and free antibodies co-exist with the adsorption of multilayered antibodies. Except for the first and the second layers, the loosely bound ones will desorb, thereby reducing the stability of the conjugates over time.[23,141,142]

Chemisorption of bioreceptors

To stably anchor bioreceptors on nanomaterial surfaces, several hetero-bifunctional ligands (linker) that included thiols or disulfide and another functional end group for a bioreceptors conjugation can be used.[142] In this context, several strategies have been reported which include chemical modification of bioreceptors and nanomaterials. In the case of chemical modification of bioreceptors, several numbers of the process have been established for chemisorptions which include the generation of sulfhydryl groups from amino groups and carbohydrate groups, the formation of sulfhydryl groups from disulfide reduction, the formation of disulfides *via* the nucleotide-binding site, etc.[101,142] On the other hand, the nanomaterial modification includes modification/functionalization of carboxyl or N-hydroxysuccinimide (NHS) ester groups, selection of fragment crystallizable (Fc)-binding proteins, etc. Similarly, in advanced into this mentioned strategies, including chemical modification into both bioreceptors and nanomaterials such as DNA/bioreceptors streptavidin complex, DNA tagged antibody conjugations, AuNPs/protein G-DNA conjugates, etc. through various reactions and strategies, namely Michael addition, reductive amination, high-affinity association, click chemistry, etc.[23,101,142] In 2018, hetero-functionalized PEG was used as a ligand to bind antibodies with AuNPs and to make the surface of the nanoparticles stable in physiological conditions.[143] Yet in this pioneering work, the researchers worked on the AuNPs surface with heterobifunctional PEG (COOH-PEG-SH) to stabilize and interact with antibodies. The application of thiol to the AuNPs surface by oxidative means initiates the thiol-Au reaction and lowers the hydrogen. The collection of X-ray photoelectron spectroscopy (XPS) spectra and zeta-potential measurement were used for the estimation of functions of PEG-coated AuNPs (PGNPs) from Bare AuNPs. The monoclonal antibody has been bound on the AuNPs surface by forming chemical connections between main amine (NH_2) groups and the carboxylic acid (COOH) group following the PEG-ylation of AuNPs by using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC)/NHS chemistry (carbodiimide chemistry). The PEG containing COOH groups (active functional groups) interacting with residual primary antibody NH_2 groups that are converted into reactive NHS-ester groups. This helps an antibody to respond to over one AuNPs and therefore, cause AuNPs-Antibody cross-linkage.[143] Silica nanoparticles have been designed to generate the amino-surface-derived silica nanoparticles using a 3-aminopropyltriethoxysilane solution. Besides, the Au-layered surface of 4,4-dithiodibutyric acid has been introduced, enabling the formation of 4,4-dithiodibutyric acid self-assembled monolayer (SAM). After that, the silica nanoparticles' amine surface was activated using EDC and introduced to the activated SAM. The EDC was used to activate the 4, 4-Dithiodibutyric acid SAM carboxylic groups, which formed esters between the amino groups of the nanoparticles and the carboxylic SAMs. Eventually, protein G has been immobilized as an antibody-bound substrate that takes advantage of the high affinity of antibodies between protein G and FC region of antibodies.[144] In another research work, the Au-coated core was functionalized with carboxyl groups via 11-mercaptoundecanoic acid (11-MUA) followed by its activation by EDC/NHS chemistry. Tau antibodies were then immobilized on the surface, which preceded the injection of a casein buffer solution (0.5%), which covers the remaining spaces on the Au surface to block the nonspecific binding of subsequently injected molecules. After that, Tau protein antibodies were immobilized followed by blocking of nonspecific interaction using casein solution.[145] Interestingly, AgNPs were functionalized using an 11-MUA solution (in ethanol). After functionalization, the AgNPs and anti-amyloid-derived diffusible ligands (ADDL) antibody bioconjugates have been prepared using covalent linking via the EDC.[146] In 2016, the SPR gold chip surface was functionalized using 11-mercaptoundecanol (11-MUD) and 11-MUA. After that, the Tau specific DNA aptamer was covalently coupled to MUA through a 5′ end NH2 modification with EDC/NHS conjugating chemistry.[147] In another work, the chip surface has been modified using EDC/NHS covalent linking that provides the amide bond among an amine and a carboxyl group.[148] In further attempt, the carboxy-methylated dextran (CM-dextran)-functionalized SPR chip has been prepared followed by amide bond formation the antibody-conjugates were fabricated.[148,149] Yet another work has been reported that the antibody immobilization on the carboxylated surface (COOH) using the standard amine-coupling method. In this line, the COOH groups were activated with EDC/NHS linking chemistry for loading of bioreceptors.[150] A similar line

of the procedure has been reported in another work, in that authors were activated the carboxy-methylated group using EDC/NHS chemistry and then the monoclonal antibodies were immobilized on this activated surface.[151] In 2018, Kim et al. reported the antibody-AuNPs bioconjugation. In that, the AuNPs surface was modified using hetero-functionalized PEG, which helps to the functionalization of AuNPs. After that, the carboxy groups of PEG were converted to NHS esters using EDC/NHS chemistry. Finally, this NHS ester of AuNPs is connected to the amine group of antibodies.[143] The EDC and N-hydroxy-sulfo-succinimide (Sulfo-NHS) have been employed as reaction accelerators between antibody [anti-Aβ(1–42)] and protein A (an intermediated for efficiently immobilization of antibody on the surface of the bimetallic sensor chip).[152] With growing advancements in the bioconjugation method, in 2020, Springer et al. reported the AuNP's functionalization using streptavidin (S-AuNPs). Additionally, the antibody was functionalized with biotin using the biotinylation kit. Furthermore, the sensor chip surface has been functionalized with COOH- and OH-thiols for primary antibody immobilization via an amino coupling. In that, the carboxylic groups were activated using EDC/NHS linking chemistry to the covalent binding of antibodies.[153] In another study, the COOH and OH functionalized PEG coated SPR chip was fabricated by using hexaethylene glycol mono-11-mercaptoundecyl acid (HSC-11-PEG-6-COOH) and triethylene glycol mono-11-mercaptoundecyl ether (HSC-11-PEG-3-OH), respectively. Further, the OH and COOH group were activated using EDC/NHS chemistry followed by streptavidin cross-linking. Finally, the biotinylated antibodies were immobilized on the activated sensor surface chip.[154] Overall, nanomaterial-bioreceptors attachment is a challenge due to the structural features of the bioreceptors, including their form, chemical stability, and function. The output of nanomaterial-based biosensors has suffered from the impacts of the applied conjugation strategy between bioreceptors and many types nanomaterials. Throughout this portion, a short discussion has been provided to provide the reader with key components for mastering the conjugation of bioreceptors and nanomaterials via several important chemisorptions or physiosorption strategies. Definitely, it is crucial how biomaterials are connected to the nanomaterials in order to maintain the operation of the conjugates. In order to conform to a particular application, this key factor must be balanced by parameters including the relative distance between bioreceptors and suitable nanomaterial and chemical alteration of bioconjugates.

In vitro diagnosis of AD biomarkers

From its inception, the SPR based biosensor is widely used for the prognosis and diagnosis of numerous life-threatening issues.[19] A suitable biosensor is needed for the peculiarity of the AD and AD biomarkers.[33] The SPR-based biosensor has been widely used since its invention to predict and diagnose various life-threatening problems.[19] To comprehend the peculiarity of AD and AD biomarkers, an effective biosensor is expected.[34] An advanced approach has emerged in the area of AD diagnosis, according to the recently published plentiful literature.[27] The ideal SPR biosensor aims at high sensitivity and selectivity toward the AD biomarkers.[144] Several studies have been completed on the application of bioreceptors and bioconjugates in AD biomarker detection.[27] It has shown admirable sensitivity and selectivity for reported AD biomarkers.[144] Bioreceptos based nanoarchitectured bioconjugates acquire good sensitivity as well as selectivity[23] that can be explored to construct an adaptable platform for effectual AD biomarker recognition. In this part, numerous detection studies have been discussed to get details and insight. In this section, we audit the performance of SPR biosensor for the detection of Aβ, p/T-Tau protein, apoE4, enzymes, etc. based on selected essential quality assurance parameters (detection range, the limit of detection, types of nanomaterial and bioreceptors, etc.). In addition to this note that, we could not unify some of the relevant information in the table because of the lack of clarity about those important qualities attributes.

Aβ and related biomarkers detection

With growing advancements in the use of bioconjugate based SPR biosensor for AD biomarker detection, a huge amount of attention has been given to Aβ recognition.[33] Bioconjugate based SPR biosensor has been displayed a promising performance for the specific detection of Aβ. In addition to this, a different SPR based sensing strategies have been employed for the recognition of this biomarker in body fluid. Generally, Aβ (MW:4-kDa) is a polypeptide derived from a considerably larger precursor of Aβ protein i.e., APP which presents in the extracellular region. As we know the cleavage due to β-secretase at position 40($A\beta_{1-40}$) in the Aβ is the main type of Aβ, whereas large chain Aβ (42 amino acid residues) at position 42($A\beta_{1-42}$) obtained from minor cleave by β-secretase.[155] Over the past few years, it has been discovered that the form of small soluble oligomers known as ADDLs is often self-assembled in addition to amyloid plaques and that ADDLs contribute to neurological disorders related to memory.[156] The correlation of ADDLs with the causes of disease and brain pathology indicates that a reliable way to identify ADDLs within the system will provide a reliable molecular mechanism for AD diagnostics in the research lab. An ultra-sensitive system for detecting ADDLs could be built from SPR biosensors and endow the first clinical laboratory diagnosis for AD. In 2005, Haes et al. reported the L-SPR biosensor for monitoring the probable interaction of ADDLs antigen and its specific antibodies (anti-ADDL) through a sandwich assay format which provides the quantitative information of ADDLs. Consequently, it assists to understand the detailed insight of the aggregation mechanism and concentration of ADDLs. In this study, triangular AgNPs have been synthesized using mica substrate and functionalized using 11-MUA ethanolic solution followed by anti-ADDLs antibodies (polyclonal) immobilization via covalent linking. After the functionalization of AgNPs, the surface was exposed to the

ADDLs concentration followed by a fixed concentration of polyclonal anti-ADDL. The SAM-AgNPs functionalization offers admirable stability as well as produced the consistent redshift when ADDLs and secondary anti-ADDLs was exposed to the functionalized sensor surface. Since ADDL formation has been hypothesized to be involved in the early symptoms of AD, a test aimed at these sub-nanomolar levels is required. In this line, owing to the noteworthy merits of the sandwich assay, such as the specific interaction among antigen and their antibodies, it is the simplest way of studying and recognization of ADDLs like molecules. This study demonstrated that the nanoparticle size (heights: 25 nm; perpendicular bisectors: 90 nm) and sensing distance (35 nm) provides the detection limit as low as <100 fM for ADDL. The brain extract sample incubation exhibited the L-SPR extinction maximum shift of 6.7 nm (751.6–758.3 nm) which was further amplified by adding seconds anti-ADDL antibody up to 10.4 nm. In the case of the CSF sample, the small L-SPR shift (2.9 nm) was observed when an aging patient CSF sample was tested with antibody functionalized substrate, which was further amplified using a secondary anti-ADDL antibody, by 4.3 nm. In the case of AD patients, it was shifted from 18.5 to 33.9 nm upon the addition of a secondary anti-ADDLs antibody. The brain extract and CSF extract responses were found to be slightly different owing to different sizes of oligomers in CSF and brain. Due to a failure to understand thoroughly AD's molecular causes and mechanisms, tools that provide insights into the aggregate states of biological species and their probable interactions in native levels can consent to patients to monitor for AD and probably for therapeutic interactions with the target species. Ultimately, the potentials of the L-SPR biosensor should be used to consider the possible mechanism and AD diagnosis.[146] Zhu et al. have carried out a similar line of work in 2009 for the detection of ADDL. In brief, the chromium adhesion layer involved the AgNPs and glass substrate that resulted in the abnormal peak shift in L-SPR assay for nonspecific reactions. Therefore, they have used the n-propyl trimethoxysilane based passivation for the bypass of this abnormal shift. In the assay, the MUA/1-octanethiol based SAM congaing carboxylate groups offer the potential for covalent linking to biotin through EDC chemistry. Overall, this L-SPR performance confirms the elimination of the abnormal peak of the chromium layer. Therefore, passivation can effectively be used for the bypassing of the peak shift that is observed from the chromium adhesive layer.[157] Many monoclonal antibodies have been extensively established for fundamental research and clinical applications in the area of AD and are partially described in terms of the monoclonal antibodies binding kinetics with β-amyloid peptide (i.e., Aβ). In 2009, Ramakrishnan et al. divulged the binding kinetics of monoclonal antibodies [for example a peptide-capturing antibody (11A50), plaque-binding antibody (IgG4.1), two classical monoclonal antibodies (6E10 and 4G8)] to Aβ_{40} (monomeric and fibrillar) using SPR biosensor. In that carboxymethylated CM5 SPR sensor chips surface were activated through EDC/NHS linking chemistry. After that, the monoclonal antibodies were immobilized for Aβ_{40} interaction followed by adding a nonspecific antibody to avoid the nonspecific bindings. The outcomes of this study reported that the IgG4.1 (epitope specificity of Aβ_{2-10}) exhibited a high affinity for Aβ_{40} fibrils (1.5 nM) and weaker affinity to monomeric Aβ_{40} (512 nM). On the other hands, the 11A50 demonstrates a preferential affinity for monomeric Aβ_{40}(32.5 nM), whereas it didn't show a binding to the fibrillar Aβ_{40} due to the C-terminal region of fibrillar Aβ_{40} might be masked inside the β-sheet fibril structure. Due to specificity to the N-terminal region of the Aβ peptide, the 6E10 and 4G8 showed moderate affinity about 22.3 and 30.1 nM for monomeric Aβ_{40}, respectively. As a result, it furnishes the potential for new as well as faster antibody screening which can be more beneficial in AD specific biomarker detection.[158] Many studies have shown that A$\beta_{(1-40)}$ is a possible and challenging biomarker for AD in cerebrospinal blood. An ultrasensitive diagnostic device is important to diagnose AD early. SPR-based biomarker detection of Aβ biomarkers is poorly sensitive in this respect, as the smaller size of the Aβ binding induces no major mass changes. As the mass on the resonant detection surface is not significantly affected or changed, both the increase in the SPR angle and the detection limit for amyloid have no/little impact on the change in the SPR angular/angle.[19,159] In this line, the proper labeling that guarantees the specific and reliable binding capacity to the target can solve the shortcomings. Fascinatingly, metal/MNPs and bioreceptors based conjugate in analytical techniques accomplishes the above-mentioned demerits (sensitivity and binding capacity) of SPR biosensor. In this shade of light, Lee et al. accomplished the ultrasensitive detection of Aβ_{1-40} using SPR biosensor using AuNPs-antibody bioconjugate. In brief, the AuNPs-antibody bioconjugate was prepared using AuNPs (ca. 80 nm.), polyclonal Aβ antibody, and deionized water (1:3:6) and stabilized by 5% casein solution. The authors contend that there was no major improvement in the SPR angle due to the use of casein in SPR. Consequently, the use of simple immobilization of the antibody and casein for blockage showed no significant difference. On the other hand, the specific interaction among AuNPs-antibody bioconjugates (ca. 20 nm diameters) and its corresponding biomarker on the bio-surface revealed a significant difference in SPR angle (0.33 degrees), which played a crucial part in the low detection limit. Consequently, it enhanced the 10^7-fold (10 ng to 1 fg/mL) sensitivity of Aβ_{1-40} (low detection limit: 1 fg/mL). Overall, this proposed SPR biosensing assay along with AuNPs-antibody conjugates can be an excellent substitute for low molecular weight biomarker detection.[139] In 2010, Xia et al. demonstrate the simultaneous detection of Aβ_{1-40} and Aβ_{1-42} followed by signal amplification through streptavidin conjugated to an antibody (N-Terminus-Specific Antibody). Briefly, the biotinylated antibodies were conjugated with streptavidin that provides the signal amplification due to greater molecular weights of streptavidin (52,800 Da) and selected antibodies (150,000 Da). In this case, the streptavidin was immobilized on the surface of the PEG-coated SPR sensor chip through cross-linking between streptavidin and carboxyl- and

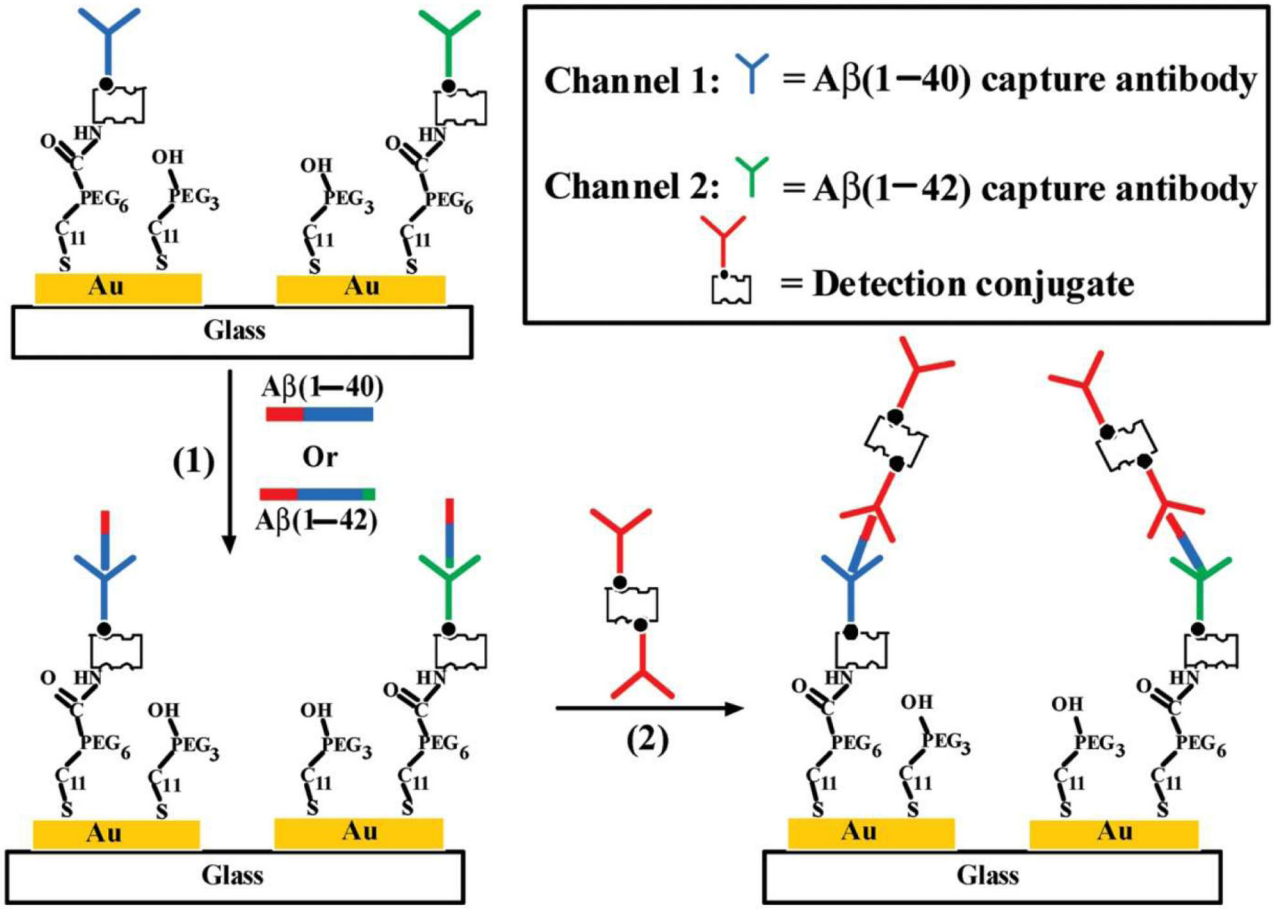

Figure 4. Simultaneous $A\beta_{(1-40)}/A\beta_{(1-42)}$ detection via SPR biosensor. Reprinted (adapted) with permission from reference [154]. Copyright (2010) American Chemical Society.

hydroxyl-terminated PEG molecules using standard amine coupling reaction (Figure 4). Ample of literature divulged that the SPR response is proportional to the mass deposited on the biosensor surface. In this case, the selected antibody on the streptavidin conjugate can detect the N-terminus of both biomarkers consequently that resulted in the signal amplification. It provides quantitative information on both biomarkers i.e., $A\beta_{1-40}$ and $A\beta_{1-42}$ in individuals as well as their ratio in health as well as AD patients CSF samples. Furthermore, the utilized protocol for signal amplification offers the selectivity to the N-terminus of $A\beta$ and consequently provides a low detection limit of about 20 pM. The $A\beta_{1-40}$ and $A\beta_{1-42}$ Concentrations in CSF were found to be 1.25 and 0.31 for healthy donors; whereas 1.13 and 0.16 for AD patients, respectively. The concentration of the ratio of $A\beta_{1-40}/A\beta_{1-42}$ in CSF was found to be 3.91 and 6.89 for healthy and AD patients respectively. In conclusion, the evidence-of-concept analysis demonstrates that SPR based biosensing can be a feasible option for simple clinical analyzes of essential neurodegenerative biomarkers.[154] In 2014, Lee et al. accomplished the $A\beta_{42}$ recognition via waveguide-couple bimetallic SPR biosensor that was based on an immobilized antibody of $A\beta_{42}$ on the surface of waveguide-couple bimetallic chip surface with the self-assembled monolayer. In that, SAM was fabricated using cross-linking material (EDC/NHS). It provides the covalent binding (amide bond) between the carboxyl and amine groups. Because of this bioassay, it provides the linear concentration range (100–2000 pg/mL) and the limit of detection as low as 500 pg/mL. Based on these findings, it has been concluded that due to specific bioreceptors immobilization on the surface of the waveguide-couple bimetallic chip and its noticeable selectivity provides the potential for detection of AD biomarkers at a low level of concentration.[148]

Peptoids are non-natural N-substituted oligoglycines used in the detection and isolation of target antibodies present in the clinical body fluids. Interestingly, peptoids are used as antigen substitutes. Due to the lack of appropriate methods for detecting biomarkers in a noninvasive biopsy, the early diagnosis of AD is difficult. Concerning this, Zhao and coauthors investigated the $A\beta_{42}$ in the serum sample using AD peptoid 3 (ADP-3) through SPR imaging (SPRi). In this assay, the binding single from anti-$A\beta_{42}$ was found to be higher in contrast to the anti-IgG, which confirmed that the ADP-3 selectively binds to the $A\beta_{42}$ in the clinical serum sample. Overall, the combination of ADP-3 microarray and SPRi offers a rapid and label free scheme for the diagnosis of AD. The increased molecule number on the surface of the self-assembled assembly may be responsible for the high affinity between $A\beta_{42}$ and ADP-3. In this regard, the electrostatic interactions between the charged amines in ADP-3 and the hydrophilic residues in $A\beta_{42}$ and the hydrophobic

interactions between the aromatic amines in ADP-3 and the hydrophobic regions in $A\beta_{42}$ may affect the interactions between ADP-3 and $A\beta_{42}$. This procedure paves the way for the early detection of AD on a blood-based clinical examination.[160] Several studies divulged that the most toxic compounds of AD are oligomeric Aβ peptides. Important for elucidating the oligomeric Aβ toxicity is the analysis of the Aβ aggregating profiles, which can be used for the therapeutic purposes of AD. Yi et al. have been developed the SPR biosensor for simultaneous recognition of Aβ in oligomers and fibrils form. Briefly, the CM-dextran-coated sensor chip was used for the individual immobilization of respective antibodies (A11 and OC) of Aβ-oligomers and Aβ-fibrils by amide bonding (EDC/NHS chemistry) between the carboxyl group and an amine group. In this SPR assay, the Au modification using CM-dextran reduces the unspecified binding of other molecules. Fascinatingly, the A11 detects the toxic oligomeric intermediate epitope of amyloidogenic proteins. Other intermediates viz. mono/di/tri/tetramers and fibrils cannot be detected. In contrast, the OC antibodies interact with fibril during SPR assay. Out of selected Aβ modulators, curcumin provided the potential inhibitor for the formation of $A\beta_{(1-42)}$ oligomer. In the case of copper (Cu^{2+}), it promotes the oligomer formation when Cu^{2+} is treated with $A\beta_{1-42}$. The $A\beta_{1-42}$ aggregation rate of the monomer to oligomer was reduced by methylene blue (MB) or the aggregation rate was increased by MB from oligomer to fibrils, which reduced the number of toxic oligomers. The findings of the reported investigation showed that Aβ aggregation and Aβ-modulator screening could be monitored using SPR biosensor as an analytical method along with rapid and detailed monitoring. It has been reported that both oligomers and fibrils monitoring were significantly improved with the accuracy of the SPR test on one single chip. Besides, chip regeneration can be achieved the reproducibility of the assay. SPR biosensor, therefore, can be a reasonable means to monitor the formation and distribution of different Aβ aggregates.[149] It is worth mentioning that the Au coated SPR sensor chip offers exceptional merits such as biocompatibility, chemical stability, and great durability. Regrettably, despite these merits, it contains low reactivity limitations due to the line width of the resulted reflectance curve. Generally, the line width of the reflectance curve was wider in the case of Au as compared to the silver (Ag) metal coating. It has been reported that the Au (10 nm) and Ag (40 nm) bimetallic SPR biosensor chip with surface-immobilized bioreceptors accomplished the sensing of $A\beta_{1-42}$. In this SPR assay, the EDC and sulfo-NHS have been used to promote the binding among protein A and antibody. Herein, protein A is generally used as a binding agent for the immobilization of antibodies on the surface of the sensor chip; it may due to the interaction of the amine terminal of the protein to the Fc region of the antibody. Ultimately, the Fab region remains free for the binding of an antigen. Thus, the immobilized antibody provides a low detection limit. The fabricated bimetallic chip-based SPR sensor offers the linear range from 50 to 1000 pg/mL, and as low as the detection limits about 54.569 pg/mL. As a result of this study, the SPR biosensor opens new doors in the biosensing field and it can be efficiently used for early diagnosis of AD.[152] With the growing interest in Aβ, highly sensitive and precise diagnostic platforms are desperately needed for its quantification. Nonetheless, there are relatively few literature reviews on original signal amplification techniques to detect AD biomarkers, so there is a growing need for more extensive bioconjugates as well as specific bioreceptors along with nanomodified material studies in detecting Aβ (or AD biomarker).

Tau protein detection

In 1975, a thermally stable protein named "Tau protein" (MW: 50–65kDa) promoted assemblies and preserved the structural integrity of microtubules that enables axon outgrowth and controlled the transportation of vesicles and organelles has become isolated.[161] Amusingly, Tau proteins are abnormally hyper-phosphorylated in the brains of persons with AD. In addition to this, Tau protein is phosphorylated with more than 20 residues, many of which are serine/threonine-proline sites (but not all).[162] In 1993, the first study was released by CSF containing Tau as an AD biomarker.[163] More specifically, Tau levels have increased in CSF in AD people in comparison with age-assigned controls, presumably by the degeneration or build-up of neuronal and axonal knots. In this line, a 195 pg/mL Tau cutoff in CSF can distinguish accurately between clinically diagnosed AD cases with 89% specificity from controls.[144] Various methods are used in Tau protein diagnostics and most of these suffered from different types of limitations such as sensitivity and selectivity problems.[144] Out of several types of a biosensor, the L-SPR-based biological sensors are becoming more common because of the remarkable benefits in biosensing of AD biomarkers i.e., Tau protein. In 2008, Vestergaard et al. divulged the L-SPR based detection of Tau protein using an affinity-based strategy between Tau protein and monoclonal anti-Tau antibody. In this study, the silica nanoparticles were activated using 3-aminopropyltriethoxysilane. The EDC has been furnished the ester bond formation among SAM an activated carboxyl group and nanoparticles containing amino groups. After that, the nanoparticles layer was coated using Au. Further, the L-SPR biosensor chip surface was chemically modified using 4,4-Dithiodibutyric acid and EDC, which provides the potential for SAM formation and its functionalization, respectively. Then, protein G (antibody-binding substrate) has been immobilized on the functionalized surface that provides the high affinity between protein G and the Fc site of the antibody. This binding offers the upright orientation to the selected specific antibody, which gives the admirable interaction with a specific target. The present study accomplished the low limit of detection as low as 10 pg/mL. In this regard, it has been confirmed that the developed L-SPR biosensor showed notable specificity as well as selectivity for the Tau protein. They considered and justified the fact that Tau detection using the mentioned L-SPR immunochip does not interfere in the presence of bovine serum albumin (the most common

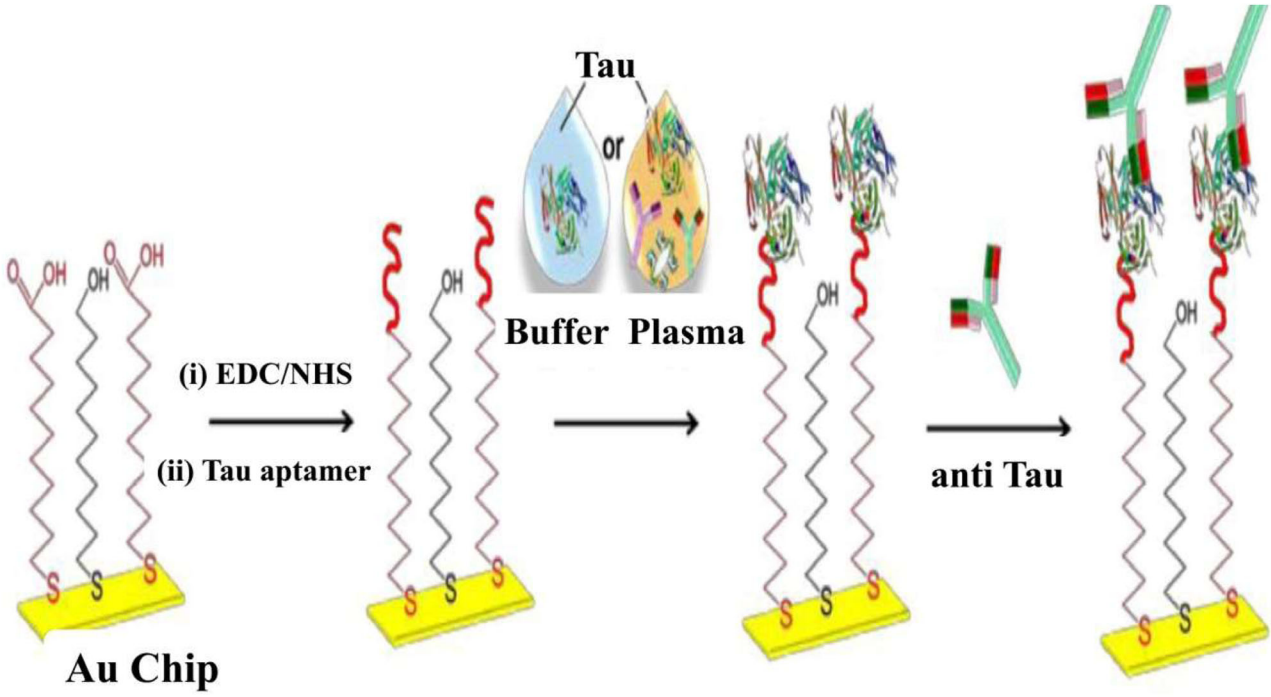

Figure 5. Schematic representation of Tau protein detection using SPR biosensor using DNA probes. Reprinted (adapted) with permission from reference [147]. Copyright (2016) American Chemical Society.

protein component in plasma, CSF). It benefits from the direct (labelless) ELISA-based assays, including uses only one antibody, could recognize a single sample at a moment and can recognize up to 300 specimens per chip multiplexing, offering versatility, and better performance than ELISA systems, etc. Therefore, it provides the promise of an economical and high-performance platform for researching Tau interactions with other proteins and/or possible pharmaceutical candidates.[144] The ability to specifically detect Tau protein and much other in human plasma/body fluids at different concentrations that are appropriate to clinical use remain a major barrier to the advancement of AD diagnostic testing. Authors have been designed a special Tau protein-specific DNA aptamer (5′ amine-modified DNA aptamer specific to Tau)/antibody (Tau antibody) pairing for the very first instance and applied this research to the multi-channel SPR framework in human plasma at fM levels for rapid screening of recombinant human Tau 381 full-length protein (Tau). It has been demonstrated that the Tau protein can bind to the single as well as double-stranded DNA. In this study, the 11-MUD and 11-MUA mixed monolayer functionalized SPR sensor chip was subjected to the covalent coupling of Tau specific DNA aptamer (Figure 5). In this line, the 5'end NH2 modification and 11-MUD are linked via EDC/NHS binding chemistry. The SPR detection signal is further enhanced by flowing anti-Tau across the chip surface, leading to the creation of the surface sandwich complex. The monolayer formation has been avoided the nonspecific reaction. In addition to this, the ratio of 11-MUD and 11-MUA controlled the measurement dynamic range, whereas the antibody concentration offers no noteworthy improvement in the SPR signal at anti-Tau concentrations more than 100 nM. Therefore, at 100 nM concentrations, the fM Tau protein concentrations were tested that provide as low as 60–170 fM Tau protein concentration in normal patient plasma. The spiked and non-spiked series analysis of Tau protein provides the 50 fM and 35 fM Tau concentrations, respectively in the account of 60% 11-MUD. Similarly, 54 fM and 52 fM were found to be for the spiked, and non-spiked series analysis of Tau protein provides the 50 fM and 35 fM Tau concentrations, respectively in the case of 60 in the account of 40% 11-MUD. Consequently, the use of multiplexed SPR may begin to expand for medical diagnosis in biofluids, as more significant disease biomarkers are identified and the appropriate nucleic acid aptamers have been chosen.[147]

Shekhar and coauthors have been investigated the Tau protein and p-Tau 181 in clinical serum samples of AD and mild cognitive impairment patients using SPR biosensor. In this shade of light, they have collected the blood samples for analysis from 39 AD patients, 37 mild cognitive impairment patients, and 37 elderly individuals as control samples. The SPR biosensor has been designed using specific antibodies for Tau protein (goat IgG) and p-Tau 181 (rabbit IgG). Then, these selected antibodies were immobilized on the sensor chip through an amine coupling kit. The outcomes form this study revealed the Tau protein and p-Tau 181 concentration in serum were significantly ($p < 0.00001$) higher in AP patients (Tau: 47.49 ng/μL and p-Tau181: 0.161 ng/μL) in contrast to the mild cognitive impairment patients (Tau; 39.26 ng/μL and p-Tau181: 0.135 ng/μL). On the other hand, the Tau protein and p-Tau 181 concentration in serum of the control group were found to be 34.92 ng/μL and 0.122 ng/μL, respectively. It is worth mentioning that, the Tau protein and p-Tau 181 concentration of AD, as well as mild cognitive impairment patients, was

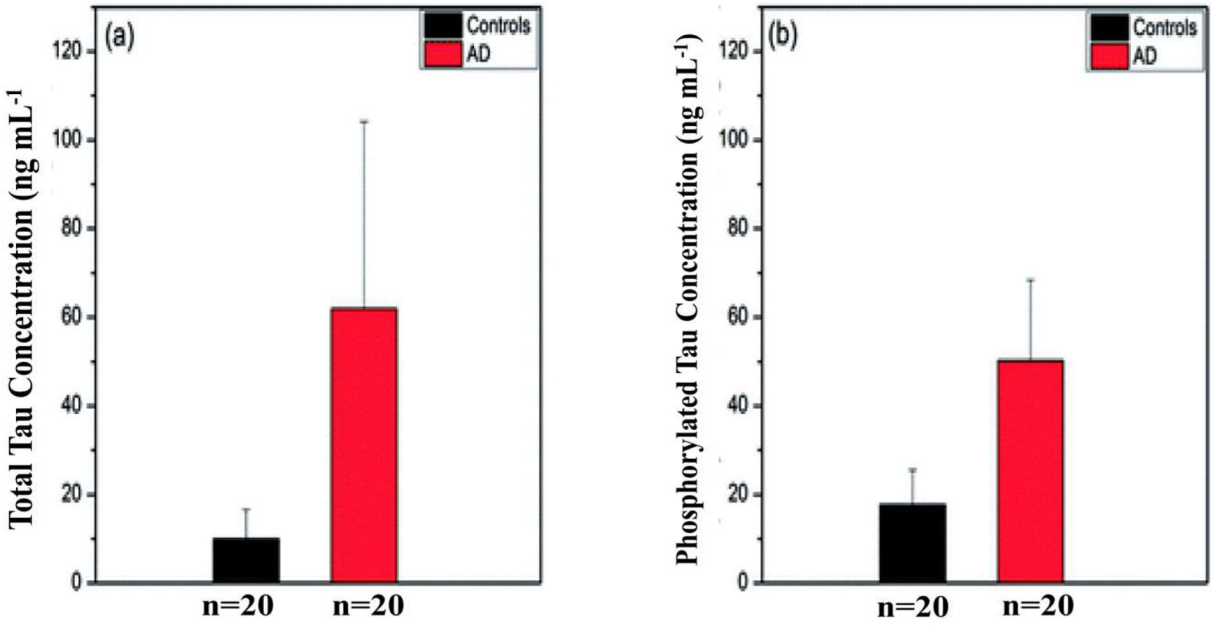

Figure 6. Average of Tau concentrations in sera of AD patients and control group, measured by SPR fiber sensors (a) T-Tau protein levels (b) p-Tau 181 levels. Reprinted (adapted) with permission from reference [145]. Published by The Royal Society of Chemistry.

reported first time in the present investigation. Overall, the cutoff values of the above-mentioned biomarkers in AD and mild cognitive impairment patients can be used for the prediction of AD and mild cognitive impairment as a predictive biomarker.[164] Yet another study reported that the label-free point-of-care-testing assay for detection of Tau protein and p-Tau 181 in human sera using SPR based fiber sensors and blood-based assay. Briefly, the in state of the Au-coated sensor chip, the authors have used the Au-coated multimodal optic fiber that was further subjected to the respective antibody immobilization through activation of the surface functional group of optic fiber (carboxyl group activation) by EDC/NHS linking. The study revealed that the concentration of Tau protein and p-Tau 181 in human sera (AD patients) was found to the higher as compared to the control groups. In this regard, the detection limit of these AD biomarkers using fabricated sensor was found to be as low as about 2.4 pg/mL and 1.6 pg/mL, respectively. Especially, in the case of AD patients, T-Tau proteins were exhibited in 6 times the higher concentration as compared to the control group (Figure 6). Similarly, the p-Tau 181 concentration was 3 times higher as compared to the control ones. Therefore, it could open the path for early diagnoses of AD and patient-friendly monitoring systems from the care point of view.[145]

SPR has previously been poorly used in immunosensing for Tau detection because of sensitivity limitations at the level of nM, whereas the pM clinical criteria. Bioreceptors, nanostructures (plasmonic/non-plasmonic), bioconjugates can amplify the SPR biosensor response and ultimately increase the detection limit to the appropriate sensitivity. Nano-modified material strategies primarily based on Au are commonly used in this shadow of light, but MWCNTs also have a strong interest in this field. In this pioneering work, Lisi et al. designed and developed the MWCNTs based on sandwich-based SPR biosensor bioassay for recognition of Tau protein. In this authors were designed different strategies, including direct detection assay using a primary antibody, sandwich assay by adding secondary antibodies after direct assay, and sandwich assay containing secondary antibodies immobilized on the surface of MWCNTs (Antibody-MWCNTs-bioconjugate). Secondary antibody-MWCNTs bioconjugate-based SPR biosensing assay provided the signal enhancement up to 10^2 folds in contrast to the direct detection as well as a traditional sandwich assay. In this study, the MWCNTs containing carboxyl groups were furnished with the covalent bonding for the immobilization of secondary antibodies. Finally, it provides about 125 pM detection limits. The MWCNTs-antibody bioconjugates have been configured to prepare and employ the purpose of detection of the pM level of Tau protein, and the bioassays were reversible under these conditions, with an average of 14% batch-to-batch variability. The application of the conjugation of the MWCNTs-antibody to enhance the analytical efficiency of the Tau protein detection via SPR sensing in the pM range and could thus provide new ways for creative and sensitive SPR methods.[165]

Multiplex/complex detection of AD biomarkers

Multifactorial pathways and results in changes in associated rates with AD further influence AD. Concerning this, the combination recognition of multiple lesions should be properly identified to increase the precision of the diagnosis of AD. In this line, $A\beta_{1-40}$, $A\beta_{1-42,}$ and Tau protein are landmarks/biomarkers in AD pathology and can act as a primary test and important markers in diagnosis. In this pioneering work, the highly selective detection of AD biomarkers ($A\beta_{1-40}$, $A\beta_{1-42,}$ and Tau protein) on one platform has been reported using L-SPR, which was, depends on the AuNPs shape (shape code biosensor). In this attempt, the

heterofunctionalized PEG (1 mM, MW: 2000) was used for the immobilization of antibodies on the surface of AuNPs (50 nm). The bioconjugate of antibody to carboxylated AuNPs has been accomplished using EDC/NHS binding chemistry that converts the PEG functionalized AuNPs solution containing carboxyl groups to NHS esters for immobilization of antibody via interaction with the amine group of antibodies (i.e., amide bond formation). The AuNPs was exhibited −12.93 mV zeta potential, which confirmed that citrate molecules (reducing agent) are attached to nanoparticles electrostatically. In further functionalization, AuNPs have been shown more negative zeta potential (−26.17 ± 0.64 mV) due to dense functionalization by PEG molecules on the surface of AuNPs. The negligible nonspecific binding of the developed biosensor confirmed the selectivity toward the AD biomarkers. Notably, the L-SPR assay provides a 20 times lower detection range as compared to the other detection assay. Furthermore, the linearity range was found to be in 1×10^1 fM to 1×10^8 fM. In addition to this, it provides a lower limit of detection as low as 34.9 fM, 26 fM, and 23.6 fM for Aβ_{1-40}, A$\beta_{1-42,}$ and Tau protein, respectively. On the whole, the multiplex L-SPR offers a worthy candidate for a highly sensitive, selective rapid, and simplistic diagnosis of many important/core biomarkers.[143] Recently, a major research aim is to establish techniques that facilitate understanding of molecular processes related to AD and the recognition of AD biomarkers at low concentrations in different body fluids. In 2020, Springer et al. developed a new method for complex detection of core AD biomarkers i.e., Aβ and Tau protein using SPR biosensor. In this study, the AuNPs have been functionalized using streptavidin, which offers the potential for antibody immobilization. Furthermore, the SPR sensor chip has been modified with COOH-/OH-thiols groups and using EDC/NHS chemistry, the primary antibodies were immobilized through covalent bonding. Besides, the ethanolamine has been used to avoid the non-covalent binding of streptavidin as well as to deactivate the remaining functional groups. Interestingly, the biotin and streptavidin interaction offers the association of the sensor to the AuNPs and consequently boosts the sensor response. In this attempt, the enhancement in the concentration of EDC/NHS offers a reduction in nonspecific responses from SPR biosensor. On the other hand, the EDC/NHS concentration shows an inversely proportional relationship with each other. Moreover, the development of the antibody immobilized dense layer strengthened the resistance to nonspecific binding. It provides the 1.5 pM and 0.8 pM of Tau-Aβ complex concentration levels in two healthy donors CSF (100%) fluids. This sandwich assay based SPR biosensor provides the 0.1 pM detection limit for Tau-Aβ complex in 10% CSF. The ability to quantify the Tau-Aβ complex levels is expected to allow for a more thorough study of the role of Tau-Aβ in AD pathogenesis as well as provide further mechanistic insights into processes associated with such a complicated disease.[153] Overall, the complex detection of AD biomarkers is an upcoming challenging task to research scholars in which the role of the above-mentioned biomarkers in AD pathogenesis, its mechanism in disease progress, etc. should be identified based on the concentration level in different body fluids.

Detection of apoE

The human apoE MW: ~39 kDa) is commonly involved in AD, gastric cancer, and other diseases such as cardiovascular diseases. Due to the crucial role of apoE in AD, and other above-mentioned diseases, its identification is offering the potential for early-stage diagnosis of such life-threatening health issues. Despite the indisputable benefits of science and technology, traditional methods, including ELISA for apoE recognition are tedious, costly, time-consuming, and required expensive equipment. Therefore, there is an urge to designed and developed the simplistic, socioeconomic, *in situ*, real-time detection methods along with high sensitivity and selectivity for label free detection of biomarkers, especially apoE. In 2013, Sciacca et al. reported the positive charge polymer i.e., poly (allylamine hydrochloride) coating on the negative charge surface of Ag that offers the amino groups for the fabrication of bioconjugates with an antibody. In this study, the antibodies have been immobilized on the polyelectrolyte coated sensor surface that shows the random orientation of antibodies. In another strategy, biotinylated antibodies have been linked to a neutravidin layer that enables the proper orientation of the antibody. Consequently, it provides the real-time detection of apoE within 1 h along with a 30 μg/mL detection limit. Moreover, it provides a linear concentration range from 5 to 10 μg/mL. They claimed that the surface functionalization of the sensor chip surface provides several merits such as sensitivity as well as selectivity to the small proteins also. Overall, the biotin-neutravidin approach can be used for the development of an SPR biosensor that provides the proper orientation of bioreceptors on the sensor chip surface as well as controls the surface density of bioreceptors.[166] To obtain knowledge regarding susceptibility to disease, the detection of gene variation is very important. In general, polymerase chain reaction, microarray methods, electrochemical methods are actively engaged in the detection of apoE. However, these methods involve some major limitations in diagnosis such as tedious and time-consuming processes require expert person, lower sensitivity, cross-reactivity, etc. Therefore, there is a prerequisite of simple, rapid, highly sensitive as well as a selective method for recognition of the apoE gene. Plentiful literature revealed that the SPR biosensor is a simplistic, socioeconomic, rapid, label free type of sensor. Owing to this commendable merits and aptitude of multiplexed analysis, SPRi biosensor has been majorly chosen for the single nucleotide polymorphism and DNA assay. Lamentably, the SPRi suffering major issue like CCD camera limits the sensitivity of the assay. Thus, it remains a major challenge for the researchers to develop a suitable SPR biosensor for the previously mentioned assay. In this pioneering work, Yi et al have demonstrated a highly sensitive and specific SPR method for quantification of the apoE gene and genotype discrimination (Figure 7). Interestingly, the HhaI restrictive enzyme could cleavage the complementary sequences to the

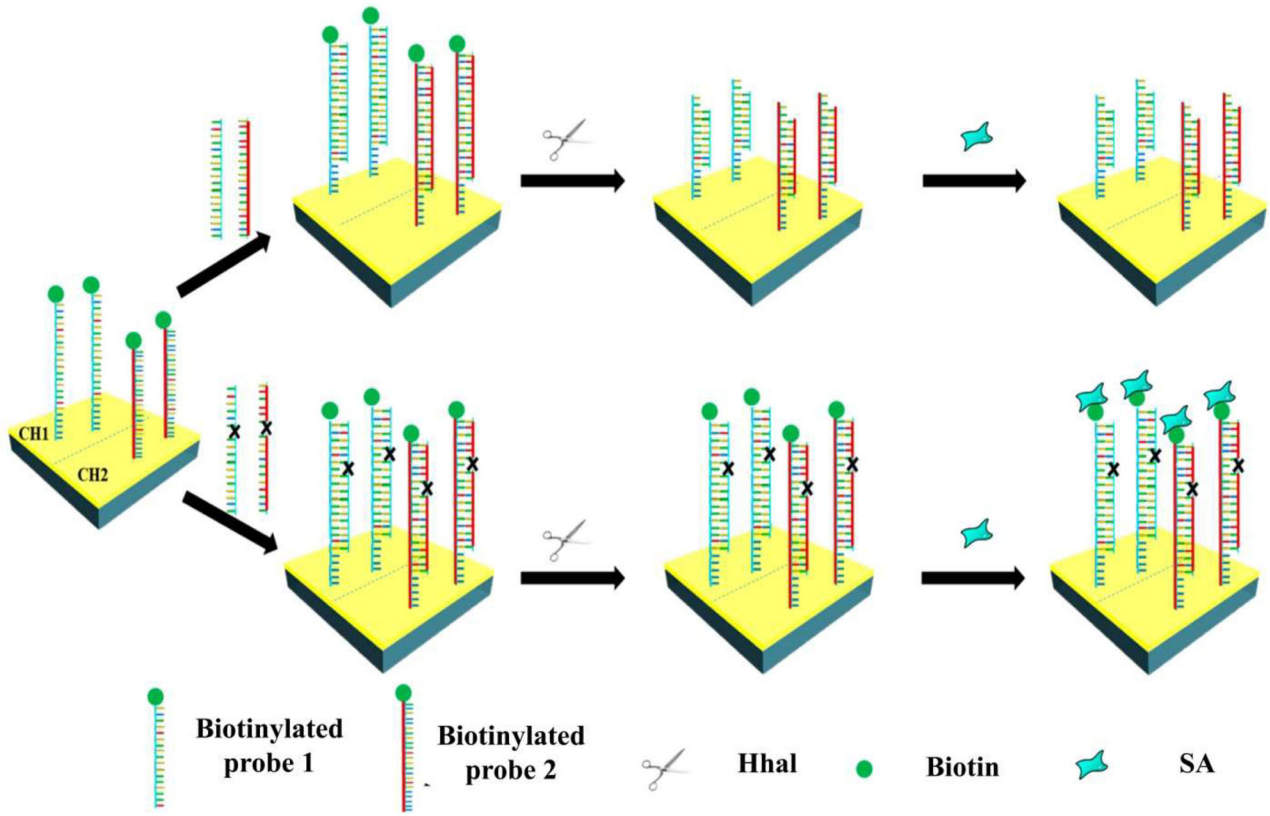

Figure 7. Biotinylated probes 1 and 2 for quantification of the apoE gene and genotype discrimination by SPR. Reprinted (adapted) with permission from reference [167]. Copyright (2018) American Chemical Society.

selective detection site of GC-GC bases upon the hybridization of pre-immobilized biotinylated probes samples. Whereas the presence of a single base mismatch like GT-GC could prevent a cleavage reaction. Briefly, the biotinylated DNA has been immobilized on the surface of 11-MUA-based SAM through EDC/NHS chemistry. The unbinding of the biotinylated fragment from the surface prevents streptavidin from interacting with a large molecular weight of 60 kDa, which leads to a smaller SPR signal. The presences of a single-base defect at the enzyme signature sites prohibit the enzyme cleavage operations and preserve the biotinylated fragment on the sensor chips. The authors state that the use of streptavidin increases the sensitivity of SPR biosensors. Additionally, biotin tag and streptavidin binding affinity is high as compared to the covalent bonding. Accordingly, it provides a lower detection limit up to 10 fM for complementary sequences and 50 fM for single-base mismatched sequences. To summarize, in 4 unamplified DNA extracts, the proposed methods can serve as a viable method for simplistic and responsive analysis of the apoE gene.[167]

Miscellaneous AD biomarkers

It is worthy of note that acetylcholine is a widely known neurotransmitter that controls people's behavior. While a deficiency in controlling acetylcholine has been related to numerous neurological conditions including AD. Thus, the cholinergic theory relates to a severe reduction in the synthesis of acetylcholine in the cause of AD. In 2018, Kant and Gupta designed and developed the fiber optic SPR biosensor for the detection of acetylcholine through the functionalization of acetylcholinesterase over Ag-tantalum (v) oxide nanoflakes multilayered optical fibers. Captivatingly, the probable mechanism involved in the detection of acetylcholine was the interaction among acetylcholinesterase enzyme and target analyte i.e., acetylcholine. This sensor provides an admirable sensitivity of 8.709 nm/μM and a good limit of detection as low as 38 nM. In addition to this, it confirmed that the sensor provides a blue shift of 44 nm with a respective change of 0 to 10 μM concentrations in resonance wavelength. In conclusion, this biosensor provides many benefits including ease of use, cost-effectiveness as well as remote sensing.[168] Many researchers have highlighted the role of mitochondrial dysfunction in AD pathogenesis. Some many lines show the Aβ association with a catalytically active mitochondrial enzyme, 17-β-hydroxysteroid dehydrogenase type 10 (17β-HSD-10; MW: 108 kDa) triggers the neuronal cell death as well as some severe mitochondrial dysfunction. K. Hegnerova and co-investigators have been developed the SPR biosensor for label free recognition of 17β-HSD-10 as well as17β-HSD-10 resulted in a synthetic peptide of 14 amino acid residues in an artificial CSF buffer. Briefly, the polyclonal antibody and Aβ were immobilized separately through SAM based alkylthiolates followed by amino coupling chemistry. In the case of the antibody-immobilized assay, the concentration range was found to be 1–1000 ng/mL. Whereas, the detection limit for the enzyme and the synthetic peptide was found to be

Table 2. Summary table of nano-architectured bio-conjugates and bioreceptors mediated SPR biosensor for in vitro diagnosis of AD.

S. No.	Biomarker	Body fluid	SPR type	Receptor	Receptor immobilization	Nanomaterials/ nanostructures	Methods of sensitivity enhancement	Sensitivity range	Limit of detection	Ref.
1.	ADDLs	CSF and Brain Extract	L-SPR	Polyclonal Antibodies	Covalent linking (EDC)	AgNPs	Sandwich assay	1 fM to 100 nM	<100 fM	[146]
2.	$A\beta_{40}$ fibrils	Lyophilized peptide form	Bulk-SPR	Monoclonal antibodies	EDC/NHS chemistry	—	Direct detection assay	33 nM to 200 nM	1.5 nM	[158]
3.	$A\beta_{(1-40)}$	$A\beta_{(1-40)}$ solution	Bulk-SPR	Monoclonal and polyclonal antibodies	Physical adsorption	AuNPs	Sandwich assay	10^0 to 10^9 fg/mL	1 fg/mL	[139]
4.	$A\beta_{(1-40)}$ and $A\beta_{(1-42)}$	CSF of healthy and AD patients	Bulk-SPR	Monoclonal antibodies	Amine coupling modification	—	Sandwich assay	0.02 nM to 5 nM	20 pM	[154]
5.	$A\beta_{42}$	$A\beta_{42}$ Solution	Bulk-SPR	Antibodies*	EDC/NHS chemistry	—	Direct detection assay	100 pg/mL to 2000 pg/mL	500 pg/mL	[148]
6.	$A\beta_{(1-42)}$	$A\beta_{1-42}$ Solution	Bulk-SPR	Polyclonal anti-Aβ (1–42)	EDC/NHS chemistry	—	Direct detection assay	250 pg/mL to 1000 pg/mL	54.569 pg/mL	[152]
7.	Tau Protein	Tau Protein	L-SPR	Monoclonal anti-Tau (anti-mouse IgG) antibody	EDC/NHS chemistry	Silica nanoparticles	Direct detection assay	10 pg/mL to 100 ng/mL	10 pg/mL	[144]
8.	T-Tau p-Tau proteins	T-Tau p-Tau proteins in the blood	Bulk-SPR	Specific Antibodies*	EDC/NHS chemistry	Fiber optics	Direct detection assay	10 pg/mL to 2360 pg/ mL 10 pg/mL to 4360 pg/mL	2.4 pg/ mL 1.6 pg/mL	[145]
9.	Tau proteins	CSF	Bulk-SPR	Monoclonal antibody	Covalent bonding	MWCNTs	Sandwich assay	125 pM to 1000 pM	125 pM	[165]
10.	$A\beta_{1-40}$ $A\beta_{1-42}$ Tau-protein	—	L-SPR	Monoclonal antibody	EDC/NHS chemistry	AuNPs	—	1×10^1 fM to 1×10^8 fM	34.9 fM 26 fM 23.6 fM	[143]
11.	Tau protein and Aβ	Cerebro-spinal fluid	Bulk-SPR	Monoclonal antibody	EDC/NHS chemistry	AuNPs	Sandwich assay	0 pM to 5 pM	0.1 pM	[153]
12.	Recombinant Tau $_{381}$	Spiked Tau	Bulk-SPR	5′-amine-modified DNA aptamer, Tau antibody	EDC/NHS chemistry	—	Sandwich assay	2 pM to 80 pM	50 fM	[147]
13.	apoE	—	Bulk-SPR	Specific Antibodies*	EDC/NHS chemistry	—	Direct detection assay	5 μg/mL to 10 μg/mL	30 μg/mL	[166]
14.	apoE	—	Bulk SPR	DNA probes	EDC/NHS chemistry	—	Direct detection assay	10 fM to 1 pM.	10 fM and 50 fM	[167]
15.	Acetylcholine	—	Fiber optic SPR	Enzyme	Direct adsorption	Ag -tantalum (v) oxide nanoflakes	Direct detection assay	8.709 nm/μM	38 nM	[168]
16.	17β-HSD-10	Artificial CSF	Bulk-SPR	Enzyme	EDC/NHS chemistry	—	Direct detection assay	1 ng/mL to 1000 ng/mL	5 ng/mL	[169]

*Types of antibodies not specified.

100 ng/mL, and 50 ng/mL, which confirmed the less affinity among antibody-enzyme and antibody-peptide. In the case of the Aβ immobilized assay, the detection limit for the enzyme in CSF buffer was found to be 5 ng/mL. On the other hand, in phosphate buffer, it was 50 ng/mL. To conclude, this sensor offers the potential of highly sensitive detection of enzymes (in the range of ng/mL) that can be used for the recognition of 17β-HSD-10 in CSF (Table 2).[169]

Summary

- SPR-centric AD biosensing has received considerable interest, owing to the amazing benefits and speedy forum for scientists to extend molecular interacting even without labeling requirements.
- The lower detection limit for specific AD biomarkers can be achievable in the range of μM to fM using nanoarchitectured bioconjugates and bioreceptors mediated SPR biosensing assay.
- Due to the remarkably strong interaction among interest AD biomarkers and preferred bioreceptors (antibodies), and bioconjugates (antibody-AuNPs) resulted in higher specificity plus remarkable sensitivity.
- SPR biosensor with multilayer of silver metal and tantalum (v) oxide nanoflakes functionalized with acetylcholinesterase provides the 38 nM detection limit for acetylcholine that exceeds many of the literature values.
- SPR biosensor with nanomodified materials (MWCNT) and affinity bioreceptors provides synergistic benefits in terms of lowest detection limit, specificity, and quantification for AD biomarker detection.
- The passivation method can be employed for the development of precise and effective SPR biosensors for early-stage detection of AD. By removing the abnormal shift associated with the nonspecific reaction, we can thoroughly assess anti-ADDLs to further clarify AD developmental mechanisms and diagnose AD effectively.
- In the case of quantification of a gene, the incorporation of streptavidin in SPR biosensor assay enhances the overall sensitivity of the assay, which offers the fM detection levels for complementary as well as single-base mismatched sequences.
- The distinct L-SPR plasmonic biosensor with AuNPs and affinity bioreceptors do not need additional methods for precisely separating and identifying markers ($A\beta_{1-40}$, $A\beta_{1-42,}$ and Tau protein from multifarious samples with fM detection limits.
- In comparison with a device comprising a particular antibody and antigen bonding on a bare golden suspension, the utilization of AuNPs-antibody complexes has increased the detection limit from ng/mL to fg/mL.
- SPR biosensor with the bimetallic (Au/Ag/Cr) chip in the intensity interrogation mode can efficiently sense various $A\beta_{1-42}$ concentrations together with critical concentration, which helps to AD diagnosis.
- SPR biosensor concluded that monoclonal antibody interactions with various monomeric $A\beta_{40}$ and fibrillar $A\beta_{40}$ structures have some fundamental variations, thus demonstrating different binding kinetics. Overall, the findings are addressed, the selection and development of appropriate monoclonal antibodies are needed for precise detection of AD biomarkers.
- The SPR data shows the first time a multi-phase interaction activity for $A\beta_{1-42}$, with two antibody-based aggregation patterns that account for the sigmoidal growth of the amyloid as a function of time.
- In SPR, the precise functionalization of the biosensor surface and the selection of AuNPs allow the pM limit of detection for the Tau-Aβ complex in CSF.
- SPR biosensor based on waveguide-coupled bimetallic chip followed by immobilization of affinity bioreceptors offers the high specificity toward the AD biomarkers.
- A combination of SPRi and ADP3 peptoid offers the highest potential for the detection of AD biomarkers in serum due to the binding of ADP3 to the $A\beta_{42}$ in serum that opens the new domain for blood-based test for diagnosis of AD.
- SPR fiber biosensor (without fluorophores) with immobilized antibodies can detect the Tau proteins (blood-based immunoassay) namely T-Tau proteins and p-Tau proteins up to pg/mL detection level.
- SPR biosensor is capable to detect the 17β-HSD10 peptide (or complete enzyme) using $A\beta_{40}$-immobilized sensor up to ng/ml levels, which confirmed the high binding affinity of $A\beta_{40}$ to 17β-HSD10 enzyme. Additionally, the $A\beta_{40}$-immobilized sensor provides a higher sensor response than the polyclonal antibody-based SPR biosensor.
- Comparative measurement of AD biomarker (Tau protein) using ELISA and SPR conclude that the SPR based sandwich assay is a more sensitive alternative.

Future prospects

From its inception, AD is a worsening and often untreatable illness with a gradual onset and steady development over a long period, resulting in increased impairment levels. Since the prevalence of AD is projected to increase exponentially in the next decades, it is important to develop analytical technologies to diagnose AD biomarkers sensitively, accurately, and economically. Besides, in terms of increasing the overall effectiveness of disease-modifying drugs, an early analysis of alterations in AD biomarkers levels contribute to wise choices and the conception of good treatment options. Concerning this, the biosensors are efficient analytical instruments that convert biological recognition phenomena into electric, thermal, or optical signals on physical or chemical or biotransducers. This discipline was one of those most intelligently studied during recent decades due to its admirable sensitivity and selectivity linked to the simple, rapid, and cheap assessment of analytes. Until now, many SPR biosensor based bio-sensing methodologies have been implemented with different substances to detect AD biomarkers. Unfortunately, the normal limit of detection is also an obstacle despite significant advances in the sensing of AD biomarkers by biosensors, since the acceptable concentration of

the AD biomarkers in body fluids is at nM-pM. In this shade of light, the role of the bioconjugate, as well as bioreceptors mediated nanoarchitectured SPR-based biosensor for the recognition of AD biomarkers has gathered much attention. Interestingly, these SPR-based biosensors are cost-efficient analytical instruments for fast screening with the desired goal. Nevertheless, the use of high sensitivity and specificity of bioconjugates and nanoarchitectured bioreceptors in SPR-biosensors will increase the recognition value of AD biomarkers. In this regard, numerous types of bioreceptors and bioconjugate-based SPR biosensors have been established. As tools to boost selectivity and sensitivity, different nanomaterials for the bioconjunction could be implemented. In addition to this, it will soon be able to make commercial use of the SPR based advanced biosensor for the diagnosis of AD with a comprehensive continuous study. Based on the present review, a few other meaningful dilemmas can be highlighted in the advancement of regularly scheduled and customizable biosensing platforms since these devices typically face some problems of reproducibility and stability in biological fluids. Despite notable development in biosensing, most of the AD biomarker detection studies have been performed in the mixture of biomarker and buffer samples or real spiked/artificial samples. Furthermore, the AD biomarker concentration in various body fluids in the respective AD patient should able to confirm the stage of AD. Additionally, the difference between AD and other related diseases based on AD biomarker concentration via SPR biosensor is a prerequisite for the AD effective treatment. For this motive, there is a major challenge to design the advanced strategies in SPR biosensors to overcome these mentioned problems. Despite revolutionary advances in SPR, there is a continuous drawback of lack of cross-validation through the academic labs, cohorts, methodologies, and industrial laboratories. Finding the positive cutoff and the specificity and sensitivity for the detection of AD biomarkers is also important in further research and future longitudinal studies. The high sensitivity and selective identification and quantification of a group or complex AD biomarkers in body fluids are also one of the major tasks for researchers. As per literature, bioconjugate and nanoarchitectured bioreceptors offer a pivotal role in resolving these above-mentioned problems. This in turn to develop the highly sensitive and selective SPR biosensor based on bioconjugates and nanoarchitectured bioreceptors for early detection of AD biomarkers. It can open a new path for early-stage AD management. Besides, depending on this complex sample analysis, AD pathogenesis and mechanistic insight can open a new path for AD treatment. A lack of knowledge of early fundamental pathological mechanisms stops progress in the advancement of successful treatments for AD. Systems biology includes numerous technologies, such as metabolomics; genomics, transcriptomics, epigenomics, proteomics, etc. Especially, metabolomics is the current system biology technique which includes targeted/untargeted metabolomics, lipidomics, and fluxomics, etc. to quantify small molecule metabolite levels in clinical, biological samples viz. CSF, brain tissue, plasma, urine, cells, and saliva among others.[170] Fascinatingly, metabolomics provides a great opportunity for neurodegenerative disease prognosis and diagnosis, as the metabolome of a person represents changes in genetic, protein profile, and transcript, as well as environmental impact. Advances in metabolomics have shown the importance of dynamic changes related to AD development, which underlines difficulties in designing effective therapeutic approaches. The mass spectrometry-based metabolomics provides greater sensitivity and the potential to quantify a wider spectrum of metabolites.[171] Paglia and colleagues claimed that the application of metabolomics approaches able to distinguish AD from control.[172] Metabolism in various tissues and fluids in AD patients suggests a systemic aspect of the disorder, along with changes to the brain. This illustrates the possible use of metabolomics in the study of pathophysiological processes of living patients. Metabolomic study of mild cognitive impairment and AD in biological samples reported metabolic changes related to pre-clinical and clinical AD. The results indicate that biomarkers based on metabolomics can be utilized to enhance the diagnosis of disease.[171] In the future, Metabolomics profiling together with a pathway analysis might help to recognize the basic mechanisms of AD and other neurodegenerative injuries, enabling the implementation of new blood-based AD biomarkers for precise prognosis, diagnosis, and regulation of overall therapeutic efficacy. In future, metabolomics derived blood based AD biomarkers can be open new avenue for early state diagnosis of AD using SPR biosensor along with high sensitivity and selectivity.

Concluding remarks

As the prevalence of AD has increased in recent years and no cure is conclusive, early detection is needed to ensure that it does not worsen. Owing to this, there is a need to develop a suitable biosensor that can offer the potential for early detection of AD. This review summarized the literature data on AD and its biomarkers sensing through SPR biosensor, with a focus on bioconjugation using bioreceptors and nanomaterials. The first part of the review comprehensively covered the AD and related core biomarkers which provide insight into AD prevalence, types of AD biomarkers, and technical developments for AD diagnosis. A further section of this review highlighted the fundamentals, types, and the importance of biosensors, especially the SPR biosensor. Further, the third part of this review depicts the rays on different bioreceptors utilized for the designing of AD biomarkers, which provides the insight of bioreceptors including merits, demerits that assist to select the appropriate bioreceptors for designing the biosensor with high sensitivity and selectivity. In the further section, we have reviewed the bioconjugation concepts along with different bioconjugation techniques. In particular, we focused on the fundamental of bioconjugation, types of bioconjugation used in the AD biomarker detection through SPR biosensor that summarizes the proper immobilization via a suitable method offers the appropriate loading density of bioreceptors on the

surface of nanomaterial that helpful for the designing of high sensitivity as well as selectivity biosensor. In the last part of this review article, we have summarized the applications of bioconjugate and bioreceptors mediated nanoarchitectured SPR biosensors for AD biomarkers detection in various body fluids in the individual or complex form. The bioconjugation, as well as nano-architected bioreceptors based SPR biosensor, endowed with the high potential for sensitivity and selectivity enhancement for AD biomarkers (viz. Aβ, Tau protein, apoE, 17β-HSD-10, etc.) detection in body fluids. Nanoarchitectured bioconjugates and bioreceptors mediated SPR biosensing assay provides the low detection limit for specific AD biomarkers in the range of μM to fM. The waveguide-coupled bimetallic chip followed by the immobilization of affinity bioreceptors offers high specificity toward the AD biomarkers. There is a need to developed suitable SPR strategies for the detection of NFL and other metabolomics-derived blood-based AD biomarkers. In conclusion, the identification of AD biomarkers in combination with SPR biosensors evolves parallel to the incorporation of different nanoarchitectured bioreceptors, bioconjugates for the testing of AD biomarkers.

Conflict of interest

The authors state that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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