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REVIEW

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Nanomaterial-based Optical and Electrochemical Biosensors for Amyloid beta and Tau: Potential for early diagnosis of Alzheimer's Disease

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ABSTRACT

Introduction: Alzheimer's disease (AD), a heterogeneous pathological process representing the most common causes of dementia worldwide, has required early and accurate diagnostic tools. Neuropathological hallmarks of AD involve the aberrant accumulation of Amyloid beta (Aβ) into Amyloid plaques and hyperphosphorylated Tau into neurofibrillary tangles, occurring long before the onset of brain dysfunction.

Areas covered: Considering the significance of Aβ and Tau in AD pathogenesis, these proteins have been adopted as core biomarkers of AD, and their quantification has provided precise diagnostic information to develop next-generation AD therapeutic approaches. However, conventional diagnostic methods may not suffice to achieve clinical criteria that are acceptable for proper diagnosis and treatment. The advantages of nanomaterial-based biosensors including facile miniaturization, mass fabrication, ultra-sensitivity, make them useful to be promising tools to measure Aβ and Tau simultaneously for accurate validation of low-abundance yet potentially informative biomarkers of AD..

Expert opinion: The study has identified the potential application of advanced biosensors as standardized clinical diagnostic tools for AD, evolving the way for new and efficient AD control with minimum economic and social burden. After clinical trial, nanobiosensors for measuring Aβ and Tau simultaneously possess innovative diagnosis of AD to provide significant contributions to primary Alzheimer's care intervention.

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Alzheimer's disease; amyloid beta; tau; diagnosis; nanomaterial; electrochemical biosensor; optical biosensor

1 Introduction

According to the recent report in September 2020, there are approximately 50 million people living with dementia and around 10 million cases surge to record per year in the world, even is forecasted to reach 82 million in 2030 and 152 million in 2050, in which 60–70% contribution of Alzheimer's disease (AD) [1]. AD is a multi-faceted neurodegenerative disease in which synaptic dysfunction is part of the fundamental contributors to pathogenesis in patients [2]. This disease is characterized by the presence of amyloid plaques comprising Amyloid beta (Aβ) and neurofibrillary tangles (NFTs) formed by the polymerization of phosphorylated Tau protein. These formations cause brain cell functional disorders, destroy connections among neurons, and ultimately cause severe symptoms in patients, such as memory loss, inability to perform simple tasks in daily life, problems with communication, and unpredictable behavior [3,4]. To attenuate these severe consequences, diagnostic methods that can recognize AD before the onset of clinical symptoms are essential for developing effective treatments at the earliest stages.

For earlier diagnosis of AD, neuroimaging and lab tests are the most popular methods [5]. Brain imaging using various techniques such as magnetic resonance imaging (MRI) [6], functional MRI [7], photon emission computed tomography [8], positron emission tomography (PET) [9], and single-photon emission computed tomography [10] are prospective approaches for determining alterations in the brain. Lab tests allow for several types of analysis, including genetic tests, cerebrospinal fluid (CSF) analysis, and the detection of biological markers [11]. However, these conventional approaches typically require time-consuming processes and can yield less sensitive results, causing false-positive diagnosis [12]. Therefore, it is crucial to develop more effective detection methods that provide precise information regarding the occurrence and progression of the disease in a manner that requires minimal time and cost.

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Notably, Aβ and Tau are considered to be the most promising pathological biomarkers for early detection of AD [13,14]. Numerous techniques have been developed for biomarker quantification; these include, but are not limited to, individual diagnostic tests, mass spectroscopy (e.g. Congo red staining [15] and Fourier transform ion cyclotron resonance [16]), immunoassays (e.g. Enzyme-linked Immunosorbent assay (ELISA) [17] and immunoprecipitation [18]), molecular imaging (e.g. fluorescent microscopy [19]), probe-based assays (e.g. quantum dot nanoprobes [20]), electrochemical techniques [21], mass spectrometry (e.g. gas chromatography-time-of-flight mass spectrometry [22]), chromatographic assays (e.g. immobilized metal affinity chromatography [23]), and synthetic analyses (e.g. meta-analysis [24,25]). However, these techniques have several undesirable limitations such as the need for expensive instruments, low sensitivity, non-environmental-friendly qualities, and instability of certain reagents. To overcome these drawbacks, recent studies have widely investigated nanomaterial-based biosensors for monitoring diseases and therapeutic response through the measurement of fluid biomarkers [26]. Interestingly, the combination of advanced nanomaterials with biosensors for the detection of simultaneous AD biomarkers in the early diagnosis holds promising potential to improve sensitivity, specificity, and screening time. Optical and electrochemical nanobiosensors exhibit advantages in direct measurement of the targets with miniaturization of complicated devices, high specificity, and sensitivity, even with small volume and low concentration target samples [27,28]. Therefore, advanced nanomaterial biosensors for the detection of simultaneous Aβ and Tau have attracted the interest of researchers to accurately diagnose AD at the earliest stage.

The capability of early AD diagnosis holds enormous potential to not only offer efficient therapy but also reduce burden in economic as well as social impact. To further investigate the clinical potential of nanomaterial-based biosensor for Aβ and Tau, this review highlights the critical pathological mechanisms regarding Aβ-Tau synergistic interaction that results in the development of AD and outlines the latest developments in nanomaterial-based optical and electrochemical biosensors used to quantify Aβ and Tau that could be applicable for AD diagnosis (Figure 1). Based on the advanced properties of these up-to-date nanomaterial-based biosensors, the advancements and challenges of nanomaterial-based biosensors for Aβ and Tau to become realistic clinical diagnostic tools have been deliberated to emphasize the promising potential applications in the early screening of AD.

2. Potential fluid biomarkers of Alzheimer's disease

Diagnosis at the early stage of AD is of pivotal importance for a variety of clinical reasons, as it aids in evaluating the efficacy of potential therapeutic interventions. However, various lines of evidence suggest the existence of a 'preclinical' stage of AD that can begin 10–20 years before a subject can be diagnosed and based on this, clinical diagnoses are not always reliable. Therefore, the development of quantitative assays is challenging, and accurate diagnosis requires a careful medical history and physical examinations [29]. Currently, numerous studies have focused on key biomarkers for developing more accurate diagnostic methods, and various promising proteins, such as Tau or amyloid species, have been identified for predicting AD [30].

2.1. Amyloid beta and the concept of the amyloid cascade hypothesis

Recently, various studies have provided support to several conceptually important observations that strongly underpin the amyloid hypothesis. Based on the findings in Parkinson's disease, although fatal neurodegeneration is the most severe consequence of NFT formation, they are not sufficient to generate the amyloid plaques that are characteristic of AD. Instead, the changes in Aβ metabolism and initial plaque formation occurring prior to the formation of NFTs are more critical events causing amyloid plaque generation. Moreover, increasing evidence suggests that genetic variability in Aβ catabolism and clearance may act as a major player in the risk for late-onset AD [31]. Taken together, these findings reveal that Aβ deposition is the primary influence of AD, and that this process is responsible for Tau phosphorylation and NFT formation that consequently leads to neuronal death and dementia.

Although the amyloid hypothesis remains controversial, numerous studies have pointed out that senile plaques primarily comprising accumulated Aβ peptide initiate the pathogenesis of AD [32]. The Aβ peptides are produced through the proteolytic cleavage of amyloid precursor protein (APP), which is a type 1 integral membrane glycoprotein. APP is expressed in a number of tissues, particularly in the synapses of neurons, and is produced in several different isoforms consisting of 695 to 770 amino acids in size. In homeostasis, APP plays an important role in facilitating a range of biological activities, including neuronal development, signaling, and intracellular transport, in the brain [33]. APP is generally cleaved by α-secretases derived from sAPPα in the non-amyloidogenic pathways; however, in the alternative (amyloidogenic) pathways, APP is cleaved by β-secretases and then by γ-secretases to produce a peptide consisting of 37 to 49 amino acid residues. Similarly, Aβ monomers spontaneously aggregate into different forms of oligomers that can subsequently form regular fibrils and deposit in plaques [31–33]. Previous studies have found that although Aβ40 concentrations can be detected at levels that are several-fold higher than those of Aβ42 in the CSF, Aβ42 is the principal component of senile plaques in the brains of patients with AD [34]. Additionally, Aβ40 and Aβ42 levels are thought to be indicative of the severity of the disease and to contribute to the formation of oligomers that represent the most toxic species [35]. The mechanism of Aβ aggregation *in vivo* remains unclear; however, several studies have shown that there are different and highly complex pathways resulting in oligomer formation [32].

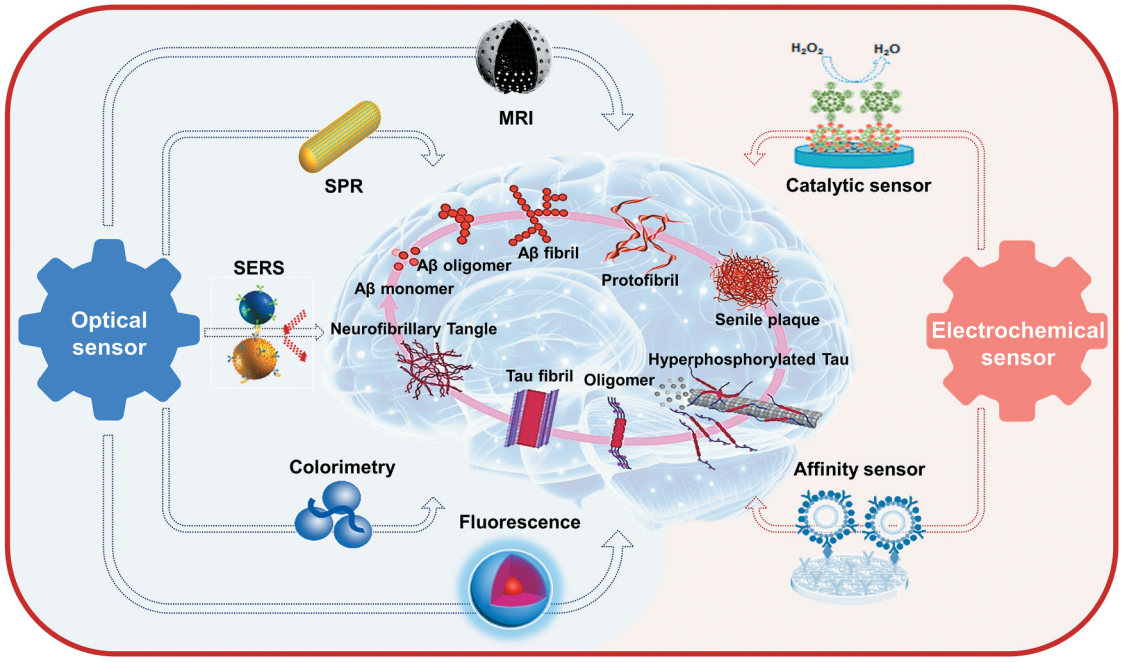

Figure 1. Cooperation between Amyloid beta and Tau pathological pathways and advanced nanomaterialbased optical and electrochemical biosensors for ultrasensitive biomarker detection in the early diagnosis of AD.

Extracellular soluble Aβ oligomers (AβO) can interact with GM1 gangliosides to induce neuronal cell death mediated by nerve growth factor receptors [32]. Aβ not only influences the neuron but also triggers the activation of both astrocytes and microglia that, in turn, generate cytokine-mediated neuroinflammation, those are the main contributors to AD progression and severity [36]. Naturally, this activation status is designed to protect the body from harm, and when needed is downregulated to reduce responses and to switch to an alternative state that functions as an anti-inflammatory phenotype in wound repair and debris clearance. However, the defective clearance of amyloid can lead to uncontrolled or prolonged neuroinflammation that is potentially harmful and can lead to chronic inflammation, cellular damage, as well as the further perpetuation of the vicious inflammatory cycle, observed in AD [37]. Based on these observations, early determination of Aβ concentration is one of the most valuable approaches for effective AD treatment.

2.2. Tau proteins and their biological function

The primary causative factor of AD and the relationship between protein misfolding and neurodegenerative disorders both remain unclear, and these have been under debate for many years [38]. In addition to senile plaques, the density of NFTs has also been speculated to be a key characteristic that closely correlates with cognitive decline in AD [39]. Regardless, unlike amyloid species that primarily exist in the extracellular environment, NFTs are intracellular and are overwhelmingly localized inside neuronal axons, and the main constituent of these NFTs is made up of the microtubule-binding protein Tau [40]. Tau is a soluble and unstructured protein in the central nervous system. The human brain expresses six isoforms of Tau that are generated by alternative splicing, contain 352 to 441 amino acids, and are subdivided into four regions and generates [41,42]. The phosphorylation in the serine residues of KXGS motif causes decreased Tau affinity, prevents it from binding to microtubules, and subsequently destabilizes the neuronal cytoskeleton. It is well established that cytoskeleton destabilization leads to disruption of Tau-dependent cellular functions, such as vesicle and organelle transport, axonal growth, synaptic transmission dysfunction [41].

Although there is much controversy regarding the relationship between NFTs and senile plaques in AD, the prevailing theory is that Tau aggregation occurs later and arises from misfolded Aβ species-dependent cellular events [43]. Under physiological conditions, Tau binds to microtubules and stabilizes the neuronal cytoskeleton [44]. However, hyperphosphorylation of Tau caused by excessive Aβ or by glycogen synthase kinase 3-β is sufficient to promote tangle-like aggregates from Tau filaments [45]. In addition, the interaction between phosphorylated Tau and Fyn can cause phosphorylation of NMDA receptor subunit 2 and enhance excitotoxicity. Furthermore, phosphorylated Tau has also been shown to interact with voltage-dependent anion channel protein 1 by interrupting axonal transport to the mitochondria, causing synaptic damage. As a result, these changes can lead to neurodegenerative disorders and can unveil the pathology of AD [46].

Table 1. Summary of nanomaterial-based optical biosensors of Aβ and Tau.

Principle	Biomaterials	Nanomaterials	Targets	Biological sample	LOD	Refs
Colorimetry	Antibody, dsDNA	AuNPs, MMPs	ADDLs	CSF	30 copies	[80]
Colorimetry	Antibody	AuNPs	Aβ42	Serum	2.3 nM	[78]
Colorimetry	-	AuNPs, AgNPs	Aβ40, Aβ42	Plasma	50 nM	[77]
Optical	-	AuNPs	Aβ40	-	-	[50]
Optical	Aβ15-20	AuNRs	Aβ40 aggregates	-	-	[76]
Optical	Antibody	Anodic aluminum oxide thin film	Aβ42 t-Tau	CSF CSF	7.8 pg/mL 15.6 pg/mL	[51]
Fluorescence	-	Cu-BTC/Tb	Aβ40	Plasma	1 nM	[69]
Fluorescence	Antibody	Magnetic bead	Aβ40 Aβ42	CSF	0.5 nM	[52]
Fluorescence	-	CDs@Eu	Aβ	CSF	0.17 nM	[64]
Fluorescence	ssDNA	GO, FAM	AβO42	Exosome, Serum	20 pM	[53]
Fluorescence	Aptamer	UCNPs, MNPs	AβO40	CSF	36 pM	[67]
Fluorescence	ssDNA	Polydopamine NPs	AβO	-	12.5 nM	[54]
Fluorescence	Antibody	MNPs	Aβ40, Aβ42	CSF, serum	-	[155]
TR-LRET	-	Eu^{3+}@PS NPs	Aβ42 aggregation	-	-	[55]
Fluorescent imaging	-	ZnO nanoflowers	Aβ	-	-	[56]
Fluorescent imaging	R7-CLPFFD peptide	brFND-35 nanodiamonds	Aβ aggregates	Fibro blast cells	-	[57]
NIR fluorescent imaging	-	Fe_3O_4@SiO_2@SLCONHR	Aβ42	Mouse brain	-	[98]
Fluorescence	Antibody, FITC	MNPs	Tau	CSF, serum	-	[155]
Fluorescence	Antibody	Fluorescent peptide nanoparticles	Aβ and Tau	Serum	-	[58]
Fluorescence	Antibody	GO, FITC	Tau	-	0.04 pM	[74]
Fluorescent	Antibody	MNPs	Tau	CSF, plasma	55 aM	[71]
Fluorescence	Antibody, aptamer	$CuInS_2$/ZnS QDs	Tau	Serum	9.3 pM	[68]
RT-QuIC	-	-	Tau fibrils	Brain homogenate	1.4 pM	[59]
Fluorescence	Aptamer	-	Tau441 Tau381 Tau352 Tau383	-	28 nM 3.2 nM 6.3 nM 2.2 nM	[60]
NIR fluorescence	Antibody	Fluorescence probe DANIR-2 c, MCAAD-3	Tau fibrils	Human neuroblastoma cells	-	[72]
Fluorescence	Antibody	MNPs, indolium fluorophore	Aβ42 Tau441 p-Tau181	CSF, serum, saliva, urine	23 fM 14 fM 34 fM	[70]
Fluorescent image	Antibody, aptamer	MNPs	Aβ42 Tau441 p-Tau181	CSF	8.4 fM 4.2 fM 3.6 fM	[73]
SPR	-	Au nano-urchins	Aβ42 fibrillation	-	-	[83]
LSPR	Antibody	AuNPs, AuNRs	Aβ40 Aβ42 Tau	Plasma	34.9 fM 26 fM 23.6 fM	[82]
LSPR	Antibody	AuNRs	Tau	Plasma	100 fM	[84]
SPR	Antibody	MWCNTs	Tau	CSF	125 pM	[87]
SPR	Antibody, aptamer	-	Tau381	Plasma	10 fM	[86]
SPR fiber	Antibody	-	Tau p-Tau	Serum	2.4 pg/mL 1.6 pg/mL	[85]
SERS	-	AuNPs@PS	HypF-N type A	-	0.1 μM	[91]
SERS	-	SiNR@AuNPs	Aβ fibril	-	-	[92]
FERS	-	Fiber	Aβ42	-	-	[61]
SERS	Antibody	MNPs, AuNPs	Tau	CSF	-	[95]
SERS	Antibody	MNPs, AuNPs	Tau	-	< 25 fM	[94]
SERS	Aptamer	AuNPs	AβO42 Tau	CSF	37 pM 0.42 fM	[90]
SERS	Antibody	MNPs@Au@GO	Aβ42 Tau		100 fg/mL	[93]
Dark field microscopy	Antibody	AuNPs	Aβ fibril	-	40 pM	[96]
STM	Antibody	AuNPs	Aβ42	-	10 fg/mL	[97]
Biolayer interferometry	Aptamer	-	Tau441	Fetal bovine serum	6.7 nM	[62]

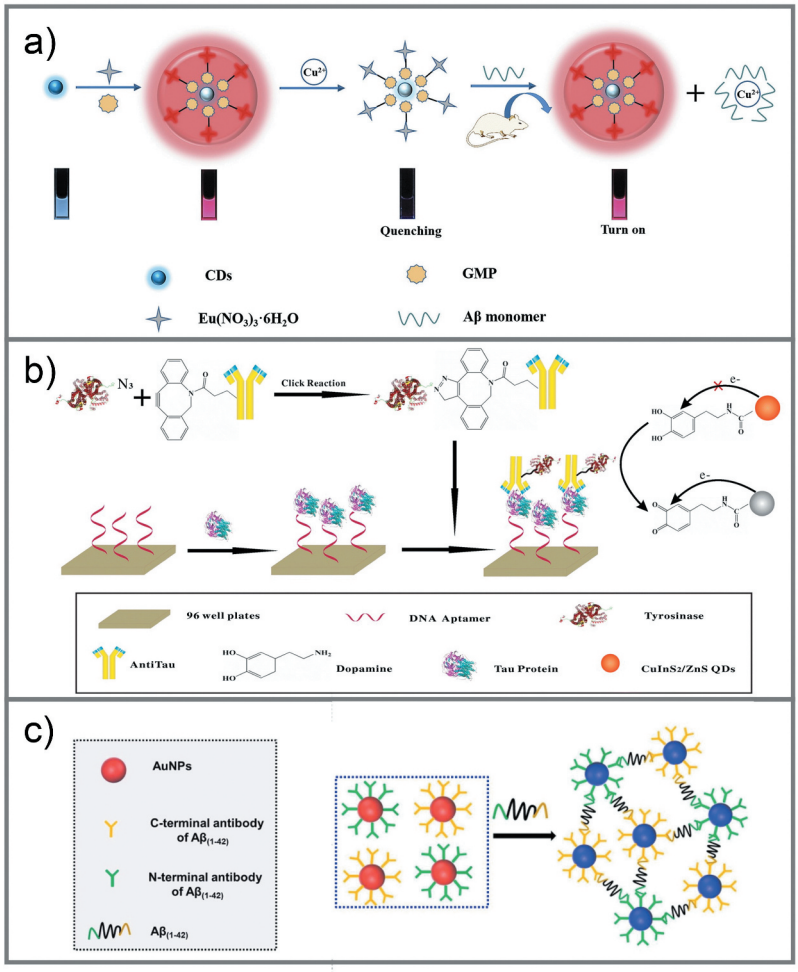

Figure 2. Schematic illustration of the synthesis of the CDs@Eu/GMP ICP fluorescent nanoparticle probes and the ratiometric fluorescent application to detect Aβ peptides based on the high affinity of Aβ toward Cu2+. Adapted with permission from Ref. [51]. B) Schematic principle of the redox-mediated fluorescent immunoassay for determination of Tau protein levels using CuInS2/ZnS quantum dots. Adapted with permission from Ref. [55]. C) Colorimetric biosensor for Aβ42 analysis based on sandwich immunoassay using AuNPs@C/N-Ab. Adapted with permission from Ref. [65].

Therefore, early detection of Tau at a low level prior to the onset of clinical symptoms may also be useful for effective AD treatment.

Current evidence suggests that the clinical symptoms of AD can begin as subtle changes that occur years before a diagnosis of dementia. For example, the accumulation of Aβ may begin as many as 20 years before diagnosis, and NFTs may occur 15 years prior to the onset of symptoms [47,48]. Therefore, it is necessary to identify and quantify these biomarkers to establish the presence of pathology at an early time-point prior to the onset of clinical symptoms.

3. Optical biosensors for Aβ and Tau biomarkers of Alzheimer's disease

Optical biosensors provide distinct advantages over conventional analytical techniques, including high specificity, sensitivity, small size, and cost-effectiveness, due to their ability to facilitate label-free, real-time, and direct detection of biological targets. A typical optical biosensor is comprised of a biorecognition sensing element incorporated with an optical transducer system, producing a signal that is equivalent to the analyte concentrations [49]. Due to their advantages, numerous optical biosensors have currently been developed to quantify the concentration of AD biomarkers (Aβ and Tau) for the early diagnosis of AD, including colorimetric biosensors, fluorescent biosensors, surface-enhanced Raman scattering (SERS) detectors, localized surface plasmon resonance (LSPR) detectors, and others. Table 1 summarizes some of the most recently developed nanoparticle-based optical biosensors used to detect Aβ and Tau.

Among the various optical biosensors, fluorescent-based biosensors are one of the most used, as they provide high-sensitivity fluorescence detection. Fluorescence-based nanobiosensors are useful and have been developed recently in the field of optical sensors [63]. Based on unique spectral characteristics, lanthanide-based infinite coordination polymers (LN-ICPs) serve as an attractive nanomaterial for time-resolved luminescence detection. Ln-ICPs exhibit large Stokes shifts, long fluorescence lifetime, and high photochemical stability, and they can readily distinguish signals of interest

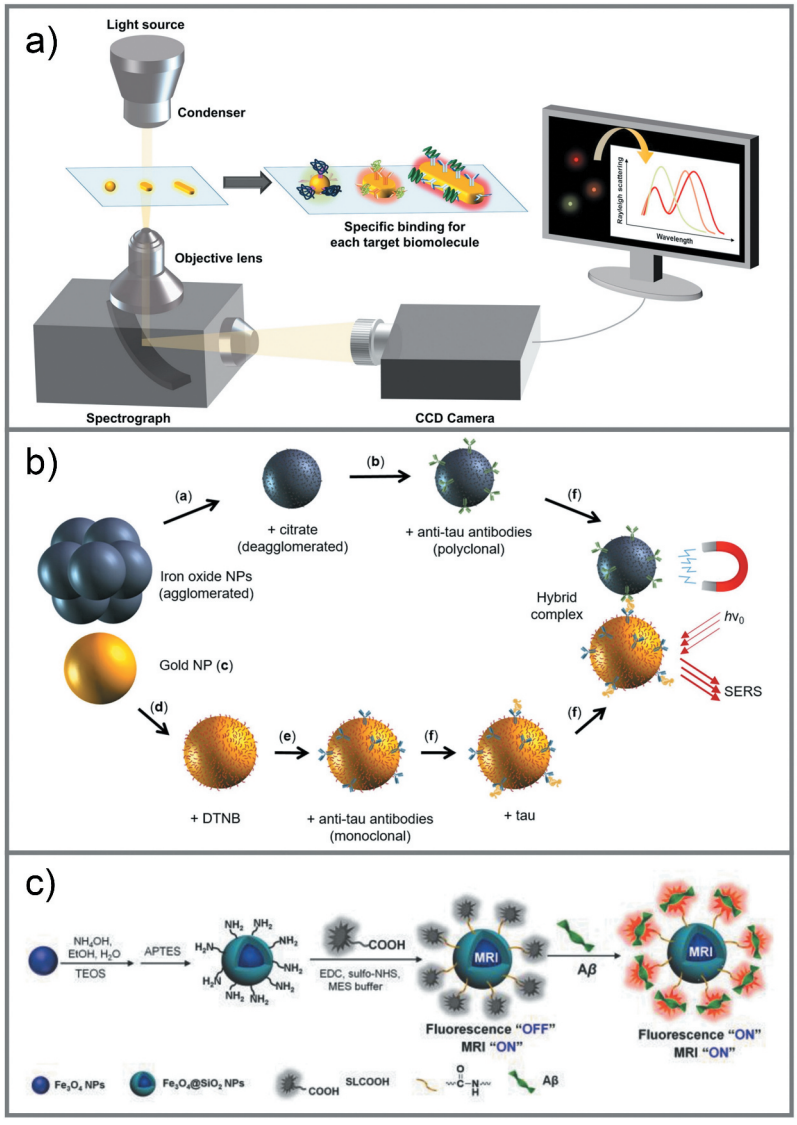

Figure 3. A) LSPR biosensor for multiple detection of Aβ40, Aβ42, and Tau using differently shaped Au nanostructures. Adapted with permission from Ref. [69]. B) SERS biosensor demonstration for Tau based on hybrid complex formation of AuNPs and MNPs immunocomplexes. Adapted with permission from Ref. [82]. C) Synthesis scheme for the fabrication of dual-modal NIR-fluorophore-conjugated magnetic nanoparticles and in vivo imaging due to signal response toward Aβ. Adapted with permission from Ref. [85]

from the background luminescence noise. A CDs@Eu/GMP ICP nanoparticle-based fluorescent probe has been recently developed to detect Aβ monomers (Figure 2A). CDs@Eu/GMP ICP nanoparticles were fabricated by exploiting the self-assembly behavior of Eu^{3+} and 50-guanosine monophosphate disodium (GMP) using carbon dots (CDs) that act as an effective antenna ligand. The interaction between CDs and Cu^{2+} could disrupt the antenna effect, ultimately leading to fluorescence quenching of Eu^{3+} while the fluorescence of CDs was retained. Due to the stronger complex formation between Cu^{2+} ions and Aβ monomers, the antenna ligand effect was restored, leading to a gradual increase in fluorescence emission at 615 nm when the Aβ monomer concentration was increased in the sample solution while the fluorescence emission at 400 nm of CDs was not affected. As a result, this inducible fluorescent nanoprobe could measure Aβ monomers in the linear range from 0.5 nM to 100 nM with limit of detection (LOD) of 0.17 nM, suggesting that it could be used as a label-free and selective probe for monomeric Aβ detection in the cerebral system [64]. Upconversion nanoparticles (UCNPs), which provide enhanced signal-to-noise ratio and possess high resistance to photobleaching, have been proposed to be a promising luminescent probe for biosensor applications [65]. Oligonucleotide aptamers, the single-stranded DNA or RNA sequences that form conformationally unique 3-dimensional structures for selective interaction with analytes, can bind to molecular targets with remarkable affinity and specificity. Aptamers can be used as convincing substitutes for antibodies due to their high

stability under hot or hazardous conditions, their ease of synthesis with high purity, and their ability to be easily modified with various molecules [66]. An immunosensor that utilized BaYF5:Yb,Er UCNPs and magnetic nanoparticles (MNPs) with aptamer functionalization to form an immune complex was developed to analyze AβO40 in artificial CSF. Aptamer-modified MNPs could rapidly separate AβO40 from the substrate solution, while UCNPs provided a background up-conversion fluorescent signal. In the presence of AβO, an immune complex containing MNPs/UCNPs was formed, resulting in a decrease in the up-conversion fluorescent intensity of UCNPs subjected to 980 nm laser excitation. This immunosensor could measure the concentration of AβO40 at LOD of 36 pM within 30 min [67]. Along with Aβ, fluorescent biosensors continue to be exploited for the detection of Tau in the diagnosis of AD. These techniques include the use of immunosensor using quantum dots [68], the coupling of nanoparticle MNPs and fluorophores, the coupling of graphene oxide (GO) and fluorescein isothiocyanate labels [69,70], MNP-based digital ELISA [71] and near-infrared (NIR) fluorescent probes for NIR fluorescent imaging [72]. A redox-mediated fluorescence immunoassay was developed using dopamine-functionalized $CuInS_2$/ZnS ($CuInS_2$/ZnS–DA) quantum dots (QDs) as the fluorescent probe for the detection of Tau in human serum (Fig. 2B). $CuInS_2$/ZnS core/shell QDs that were decorated with dopamine exhibited strong luminescent emission at approximately 750 nm. The immunosensor consists of a captured DNA aptamer of Tau and a detected antibody conjugated with a tyrosinase enzyme. Fluorescence quenching of $CuInS_2$/ZnS–DA QDs was triggered by dopamine quinone, which functioned as an effective electron acceptor transformed from dopamine by tyrosinase catalyzation of the tyrosinase conjugated antibody. The redox-mediated fluorescence immunoassay could detect Tau at a concentration range of 10 pM to 200 nM with LOD of 9.3 pM [68]. Digital ELISA for quantification of Tau was developed using MNPs. Tau was first captured by the complex MNPs@antibody and a secondary antibody-conjugated β-galactosidase enzyme. An enzyme fluorogenic substrate was added to the microarrays after the magnet-assisted loading of particles into microwell arrays was performed to achieve a fluorescent signal for the digital quantification of single molecules. Total Tau levels in blood plasma were measured at a LOD of 55 ± 29 aM, and this was 5000-fold more sensitive than commercially available ELISAs [71]. An aptamer-based multiplex detection platform for the quantification of AD protein biomarkers was developed by employing an inducible fluorophore in combination with total internal reflection fluorescence microscopy. The recognition of an aptamer-antibody pair provides high specificity for Aβ42, Tau441, and p-Tau181 detection at the femtomolar concentration range with LODs of 8.4, 4.3, and 3.6 fM, respectively [73]. An immunosensor using GO nanosheets and fluorescein isothiocyanate-labeled Tau (Tau-FITC) was investigated for its ability to detect Tau. Tau-FITC is absorbed on the GO nanosheets, leading to the fluorescence quenching effect. The binding sites of Tau-FITC on the GO nanosheets were limited due to competition between Tau and Tau-FITC, resulting in the expression of a fluorescent signal from free Tau-FITC. This platform could restrict the use of labeled secondary antibodies to allow for protein recognition and optical signal generation, thus making it simpler and more sensitive (LOD 0.14 pM) than conventional ELISA [74]. This proposed luminescent biosensors hence provide sensitive and rapid sensing platforms for the detection of Aβ and Tau toward the accurate diagnosis of AD.

Colorimetric biosensors are highly attractive for use in point-of-care detections due to their ability to detect analytes through color changes that can be easily observed by the naked eye or using simple portable optical detectors for quantitative measurement. Gold nanostructures can be easily functionalized and display different colors depending on their size, shape, and state of aggregation. Based on this, they provide an excellent platform for the development of colorimetric biosensors [75]. Gold nanoparticles (AuNPs) and gold nanorods (AuNRs) were employed in immnunoassays to measure the levels of Aβ peptides, AβO, and Aβ aggregations [76–78]. A colorimetric sandwich immunosensor specific for Aβ42 was developed based on the assembly of AuNPs, and a schematic illustration is shown in figure 2 C. AuNPs functionalized by dual anti-C/N-terminal Aβ42 antibodies were highly dispersed and exhibited a red color at an absorption peak of 525 nm. The addition of Aβ42 into the antibody-AuNP solution initiated the sandwich immunoreaction, leading to the aggregation of AuNPs as indicated by an obvious color change from red to blue. Aβ42 could be measured even by the naked eyes at a concentration range from 7.5 to 500 nM with LOD of 2.3 nM, suggesting a visual and much simpler sensing system compared to conventional ELISA [78]. Due to the extremely high affinity between Cu^{2+} ions and Aβ, cost-effective and rapid platforms for the diagnosis of AD were developed to detect Aβ using AuNPs and AgNPs [77]. AuNPs and AgNPs are efficiently adsorbed Aβ40 and Aβ42 monomers due to their large surface-to-volume ratio and electrostatic interactions, thus causing NPs to become stable and dispersed in the presence of Aβ. Cu^{2+} coordinates with the histidine residue at a position of 13 (or 14) on one Aβ and with the histidine residue at a position of 6 on the other Aβ [79] to facilitate aggregation formation of nanoparticle with Aβ in the surfaces. Label-free AuNPs and AgNPs sensor arrays could discriminate among Aβ42 and Aβ40 at a concentration range from 50 nM to 500 nM in human plasma samples [77]. A bio-barcode amplification assay was developed to detect Aβ-derived diffusible ligands (ADDLs) using a sandwich complex of antibody-immobilized MNPs with double-strange DNA and Aβ antibody-modified AuNPs. After the release of DNA from a sandwich complex, the DNA was amplified and captured on a microarray using oligonucleotides that are complementary to the DNA bio-barcode. Silver enhancement was used to intensify the signal after complementary oligonucleotide-functionalized AuNPs were applied. This bio-barcode assay could identify targets in CSF at LOD of 30 copies [80].

SPR-based biosensors hold potential in the context of high sensitivity surface plasmons excited at a metal-dielectric interface to a change in the refractive index of the dielectric. When

Table 2. Summary of nanomaterial-based electrochemical biosensors of Aβ and Tau.

Platform	Biomaterials	Nanomaterials	Targets	Biological sample	LOD	Refs
Si/SiO2	Antibody	Au-SWCNT	Aβ	Serum	1 pg/mL	[106]
ITO	Antibody	Mn: CdSe-Bi_2WO_6/CdS	Aβ	Serum	0.068 pg/mL	[131]
Glass wafer	Antibody	Si/SiO_2/Ti/Pt	Aβ	-	8.15 fM	[134]
ITO	-	AuNPs	Aβ	Mouse brain	20.7 ng/g	[137]
IDE-Au	Antibody	-	Aβ	-	10 pM	[140]
SPE	Antibody	Graphene/rGO	Aβ40	Human plasma Mouse plasma	2.398 pM	[114]
Gold electrode	Aptamer	SA-AuNPs	Aβ40	Artificial CSF	93.0 pM	[138]
GCE	Peptide	ABTS-PDDA/CNTs	Aβ42	Rat plasma Rat hippocampus	0.5 ng/mL	[107]
SPE	Antibody	CNT-Cu	Aβ42	Serum	0.4 pg/mL	[108]
SPE	Antibody	MCd/PANI/Au	Aβ42	-	0.20 ng/mL	[109]
GCE	Antibody	Au/$NiFe_2O_4$@GO-CS	Aβ42	CSF	3.0 pg/mL	[112]
GCE	Antibody	G/Co_9S_8—Pd	Aβ42	Artificial CSF	41.4 fM	[113]
Gold electrode	Antibody	GO@MNP	Aβ42	-	5.0 pg/mL	[115]
Paper-based Biopolar electrode	-	–	Aβ42, AβO42	Mouse CSF	100 pM	[122]
Gold electrode	Antibody	CSIPs	Aβ40 Aβ42	Mouse serum	6.63 pg/mL 3.74 pg/mL	[123]
IME	Antibody	AuNPs	Aβ42	Mouse plasma	-	[125]
ICE	Antibody	-	Aβ42	Human serum Human CSF	100 pg/mL 500 pg/mL	[126]
GCE	Antibody	Luminol@SnS_2 Cu-WO_3-Au	Aβ42	Human CSF	5.4 fg/mL	[127]
GCE	Antibody	$MnCO_3$/PDDA/Au	AβO42	Human CSF	19.95 fg/mL	[129]
GCE	Antibody	Fe_3O_4@PPy-Au Co-MOFs/ABEI-Au	Aβ42	Human serum	3 fg/mL	[130]
SPE	Antibody	AuNPs	Aβ42	Human serum Human plasma	100 fM	[132]
Gold electrode	Antibody	AuNPs	Aβ42	-	5.2 pg/mL	[135]
Gold electrode	PrP_{95-110} peptide	PPyCOOH/Au	AβO	Mouse brain	0.1 fM	[149]
Gold-SPE	PrP_{95-110} peptide	POPA	AβO	CHO cell cultured medium	0.5 pM	[124]
Gold rod electrode	Aptamer	-	AβO	Artificial CSF	0.03 nM	[128]
Gold electrode	PrP_{95-110} peptide	AuNPs	AβO	Serum	45 pM	[133]
Gold electrode	PrP_{95-110} peptide	AgNPs	AβO	Serum Artificial CSF	6 pM	[136]
Ni foam	-	Curcumin-Ni	AβO	Artificial CSF	0.1 nM	[139]
SPE	Aptamer	CG-Th-AuNPs	Tau381	Serum	0.70 pM	[118]
Gold electrode	Antibody Aptamer	AuNPs	Tau381	Human serum	0.42 pM	[143]
Gold electrode	Antibody	MWCNTs-rGO-CS, AuNPs	Tau441	Serum	0.46 fM	[116]
ITO	Antibody	GO-AuNPs	Tau441	CSF Serum	0.091 pg/mL	[117]
GCE	Antibody	Cu^{2+}/PTSA@rGO	Tau441	Human serum	75 fM	[119]
Gold electrode	Antibody	-	Tau441	-	-	[142]
Gold electrode	Antibody	-	Tau441	Human serum	0.03 pM	[144]
Gold electrode	Tau peptide	-	Tau441	-	1 nM	[145]
Fluorine-doped ITO	Antibody	FeOOH/Mo:$BiVO_4$	Total Tau	Human plasma	1.59 fM	[141]
Gold film	Antibody	-	Total Tau	Human serum	-	[146]
CNT	Antibody	SWCNT@Cr/Au/SiO_2	Aβ42 Aβ40 t-Tau p-Tau181	Human blood	2.13 fM 2.20 fM 2.45 fM 2.72 fM	[120]

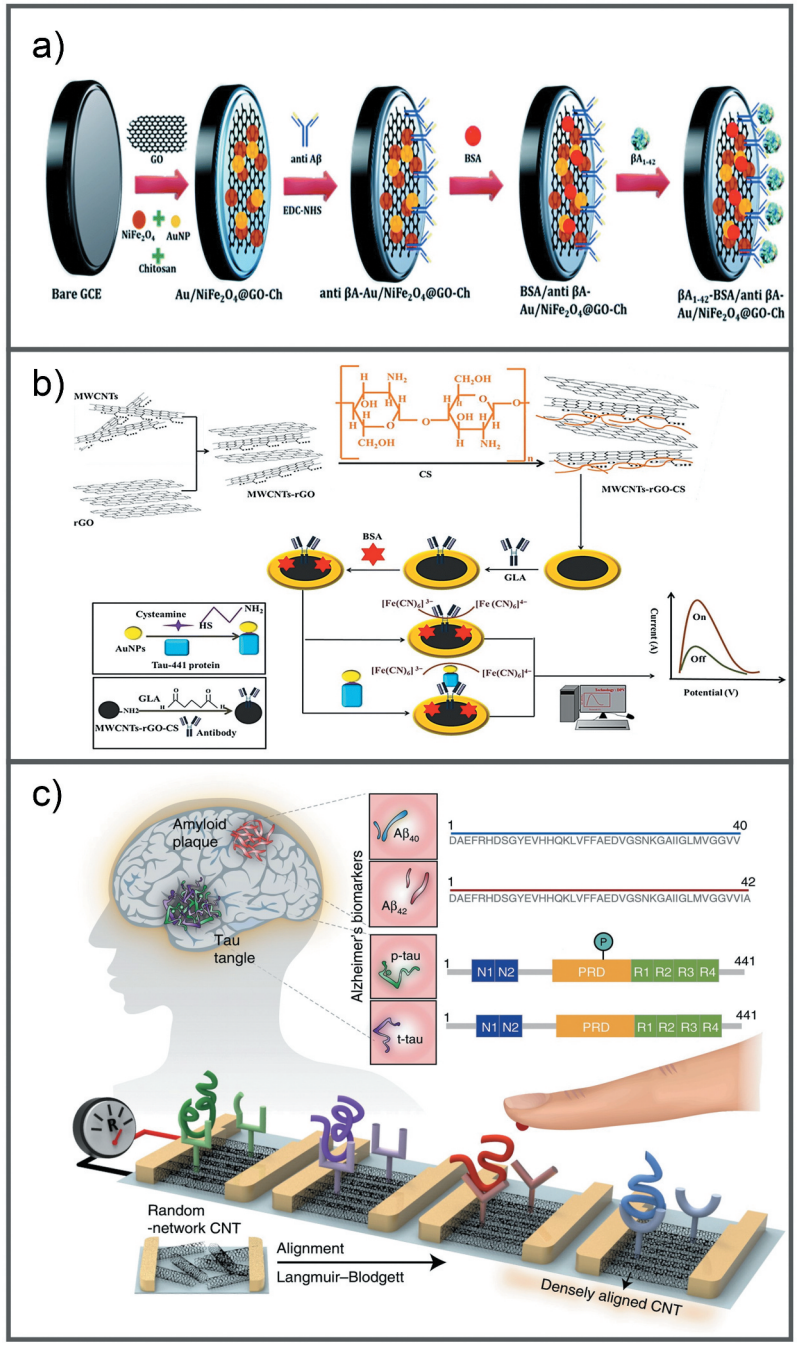

Figure 4. The procedure of the Au/NiFe2O4 nanoparticle-decorated graphene oxide-based immunosensor fabrication. Adapted with permission from Ref [113]. B) Schematic illustration of the carbon nanomaterials and gold nanoparticle-based signal multi-amplified immunosensing assay for Tau441 detection. Adapted with permission from Ref [117]. C) The preperation and detection process of multiplex densely-aligned CNTbased biosensors for simultaneous detection of Aβ42, Aβ40, t-Tau, p-Tau181. Adapted with permission from Ref. [121].

a biomolecular interaction occurs, the refractive index near the surface is replaced in a manner that is related to the concentration of the analytes [81]. To achieve higher sensitivity with lower LOD, SPR structures have been investigated using AuNPs, Au nano-urchins, and AuNRs [82,83]. Due to the observation that the plasmon resonance wavelength on the surface of the nanoparticle depends upon shape, size, and local refractive index surrounding Au nanostructures, SPR wavelength can be easily altered by modifying the shapes and sizes of Au structures without the need for any dye. Nano-urchins with sharp spiky morphology exhibit advantages over spherical metal nanoparticles by providing a larger surface area for the binding of biomarkers and a stronger localized field enhancement that provides the structure with improved

sensitivity. Due to these benefits, Au nano-urchin-based LSPR was then used to detect Aβ42 fibrillation. The fibrillation process was analyzed based on the reduction of the LSPR peak intensity [83]. Gold nanorods with guanidine hydrochloride as a chaotropic agent were employed to analyze Tau at the femtomolar levels using LSPR. Guanidine hydrochloride hinders other proteins from concealing the epitope of Tau in blood plasma, thus overcoming this obstacle in blood-plasma-based AD diagnostics by disordering water molecule networks and weakening the hydrophobic interactions between proteins. This platform exhibits highly sensitive detection of Tau in plasma despite the observation that this biomarker is present in 10- to 100-fold lower concentrations in plasma compared to its levels in CSF [84]. This immunoassay uses an SPR fiber sensor with an Au film coated in the core of a multimode optical fiber to measure Tau, including total Tau and phosphorylated Tau, at LOD of 2.4 pg/mL and 1.6 pg/mL, respectively [85]. A DNA aptamer/antibody sandwich immunoassay was used to detect human Tau381 in undiluted plasma at concentrations as low as 10 fM, representing a 1000-fold performance enhancement for SPR compared to ELISA [86]. Another sandwich immunoassay was performed for the detection of Tau using multi-walled carbon nanotubes (MWCNTs) as mass enhancers based on SPR sensors. This platform could significantly amplify signals to detect Tau concentrations as low as 125 pM [87]. To enhance the diagnostic accuracy of AD, a selective non-labeling platform for the detection of Aβ40, Aβ42, and Tau was developed using nanoplasmonic sensors that incorporate distinct LSPR (Fig. 3A) [82]. This sensor utilizes three different shapes and sizes of gold nanostructures including Au nanospheres (54 nm diameter) and Au nanorods (8.5 × 96 nm and 34.7 × 124.8 nm) in conjunction with functionalized specific antibodies. Gold nanostructures were incubated with ethanolamine to block nonspecific binding; then AD biomarkers were added and incubated for 1 hour. The plasmon shift Δλmax of the LSPR spectra was determined using a CCD camera. A one-step multiple detection LSPR system could directly quantify the concentration of AD biomarkers with LOD of 34.9 fM for Aβ40, 26 fM for Aβ42, and 23.6 fM for Tau, suggesting that this system provided a highly sensitive, rapid, and simple format for the early diagnosis of AD [82]. Although SPR has been investigated for its ability to detect picomolar concentrations of a number of biomarkers [88], only a few studies exist that examined the detection of Aβ and Tau in biofluid samples for the diagnosis of AD [82–87]. Nanomaterial-based SPR functions as the sensitive and label-free detection platform for Aβ and Tau in early diagnosis of AD with high advantages compared to conventional techniques that require binding additives or fluorescent biomarkers.

SERS has recently become a highly active research area in the field of biosensors. SERS substrates play a key element in SERS experiments. To enhance the Raman scattering signal, plasmonic nanostructure amplification has been exploited for use in optical sensor fabrication [89]. Several SERSs were reported to detect Aβ peptides, oligomers, and fibrils using nanostructures such as AuNPs [90], AuNPs@polystyrene [91], SiNR@AuNPs [92], MNPs, and GO [93]. Three-dimensional (3D) nanostructures, such as 3D Si-based SERS substrates, can increase the density of plasmonic nanostructures to supply large interspace enhancement for the detection of label-free Aβ fibrils. Aβ fibrils were detected using large-scale ordered hexagonal-packed SiNR arrays that were functionalized with homogeneous AuNPs (SiNRs@AuNPs) by receiving Raman signals at a single-fibril level. By changing the diameter of SiNRs, the resulting long decay length (>130 nm) of the enhanced electric field causes the SERS substrate to function as a zero-gap system for Aβ fibril detection. The contribution of plasmonic AuNPs to SiNRs supplied reproducible Raman signals to allow for high sensitivity in the detection of large-sized Aβ fibrils [92]. In another SERS platform, the excellent sensing properties of AuNPs were exploited. Hybrid gold-decorated polystyrene beads were immobilized with an effective metallorganic Raman chemoreceptor that was composed through the coordination of Al^{3+} ions and 4-mercaptobenzoic acid (MBA). This MBA-Al^{3+} complex supplied effective adsorption sites for oligomer interaction. The adsorption of AβO onto the metallorganic MBA-Al^{3+} complex results in characteristic changes in the SERS profile that are quantitatively proportional to the concentration of the HypF-N oligomers [91]. SERS-based magnetic sandwich immunosensors were employed using MNPs and AuNPs with DTNB as the Raman reporter to detect Tau in the femtomolar range [94,95]. The nanohybrid formation between two nanoparticles after the addition of Tau provided the SERS signal from the Raman reporter in a Tau-concentration dependent manner (Fig. 3B). The LOD of the SERS-based magnetic immunosensor was 25 fM within several minutes [94]. Furthermore, SERS has been demonstrated to be an excellent technique for the simultaneous detection of multiple biomarkers. Plasmonic nanostructures are commonly utilized to amplify the signal of the Raman reporter based on the strong local electromagnetic field enhancement near their surfaces. The SERS-based simultaneous detection of AβO42 and Tau protein was performed using 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) Raman dye-coded polyA aptamer-AuNPs conjugates. AuNPs@PAapt@DTNB conjugates are synthesized by successively self-assembling non-fluorescent Raman dyes and polyA block oligonucleotides on the surfaces of AuNPs. In the presence of AD biomarkers, the specific binding of aptamers to proteins results in the detachment of polyA block oligonucleotides from AuNP surfaces, ultimately leading to aggregation of AuNPs that exhibit significantly strong SERS signals. This platform displays excellent analytical performance regarding the detection of Tau protein and AβO42 at levels as low as 0.42 fM and 3.7 pM, respectively, within 15 min, thus allowing rapid and sensitive detection of protein biomarkers for AD diagnosis [90]. Magnetic core-plasmonic shell nanoparticle-decorated hybrid GO was developed for the selective separation of AD biomarkers from whole blood samples and subsequent measurement through label-free SERS. Due to the strong plasmonic shell of Au and the large

surface area of GO, these nanoparticles exhibited extremely high adsorption toward the target molecules to allow for sensitive SERS detection of Aβ and Tau after magnetic separation, even at the 100 fg/mL level. The high sensitivity achieved by this method primarily depends upon the strong plasmon-coupling that generates large amplified electromagnetic fields [93]. The SERS platform for detecting AD biomarkers possesses advantages over current methods, such as ELISA, LSPR, and chromatography, including label-free methodology and rapid detection using cost-effective substrate fabrication technique to provide accurate and ultrasensitive detection of Aβ and Tau for the early diagnosis of AD.

Several other optical biosensors were also used to detect Aβ through applications including dark-field microscopy [96], scanning tunneling microscopy (STM) [97], and NIR fluorescent imaging [98]. Dark-field microscopy was utilized to detect Aβ fibrils using AuNP aggregation with LOD of 40 pM, showing higher sensitivity compared to the probe dye method [96]. AuNPs-based scanning tunneling microscopy was also developed based on immunocomplexes comprising Aβ42 and antibody-conjugated AuNPs for monitoring Aβ42. The frequency of the pulse-like peak is dependent upon the surface density of the localized AuNP conjugates, which is directly correlated with the concentration of Aβ42 at range of 10 fg/mL – 1 μg/mL and LOD of 10 fg/mL [97]. Based on the strong affinity toward Aβ peptides, the acid-functionalized cyanine SLCOOH was synthesized and functionalized onto the Fe_3O_4@SiO_2 MNPs to fabricate the dual-modal nanoprobe by integrating fluorescence cyanine sensors with MNPs-based MRI probe for imaging of Aβ species *in vivo* (Fig. 3 C). Due to the strong binding interactions between Aβ species and the cyanine fluorophore that exhibited a large fluorescence enhancement at 650 nm in the NIR region and the MRI signal, this nanoprobe was successfully applied for *in vivo* fluorescence imaging with high sensitivity and selectivity to Aβ species. Additionally, MRI with high spatial resolution was used in a mouse model for real-time monitoring of Aβ species [98]. These optical imaging methods provide potential tools for early AD detection.

4. Electrochemical biosensors for Aβ and Tau biomarkers of Alzheimer's disease

Electrochemical platforms are widely favorable for use in the detection of Aβ and Tau due to the simplicity of their manufacture, low-cost, portability, and high sensitivity [99]. Typically, an electrochemical sensing system consists of two main parts: a biological recognition element (antibody, enzyme, and nucleic acid) selectively reacting with the targeted molecules and an electrode that serves as the signal transducer to convert the molecular recognition of the target analyte into an electrical signal. To maximize the signal transduction efficiency, various chemical modification methods have been applied to the electrode, including the addition of functional nanomaterials [100,101] or the fabrication of different types of nanoparticles [102,103]. Appropriate immobilization should result in high-performance and low-cost detection systems, which are typically through the utilization of proper functional materials for the electrode. Based on these modification criteria, different nanostructures for modified biosensors (summarized in Table 2) have been developed, enabling the early diagnosis of AD through the quantification of low-abundance AD biomarkers.

Carbon nanomaterial-based devices are among the most broadly applied biosensors due to their unique electrochemical properties including large functional surface area, high electrical conductivity, and low cost. Carbon nanotubes (CNTs), for example, are one of the most exciting materials used in biosensor applications due to their advantageous mechanical and electronic properties [104,105]. Numerous modifications of CNTs have been performed, including CNT fabricated with a metal semiconductor field effect transistor structure [106] and the creation of 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) and poly (diallyldimethylammonium chloride) – bifunctionalized SWCNTs (ABTS-PDDA/CNTs) [107] that exhibit the improved performance of the resultant sensing system in regard to detecting different isoforms of Aβ and Tau. A CNT-based electrode containing a copper-CNT-modified carbon surface was proposed for Aβ42 recognition in the serum samples [108]. The sensor fabrication procedure was initiated by decorating functionalized CNTs with CuO NPs to form a nanocomposite on the surface of carbon working electrode area (CNT-Cu/C-SPE). A monomeric aniline-linked plastic antibody against Aβ42 was then immobilized on the nanocomposite to obtain a molecularly imprinted polymer material (MPan/Aβ42/CNT-Cu/C-SPE). This redox probe-free sensing system possessed improvements in terms of sensitivity for Aβ42 detection in human serum samples with lower LOD (0.4 pg/mL) compared to that reported by previous studies using the same plastic antibody-based platform (LOD 0.20 ng/mL) [109].

Another carbon nanomaterial, graphene and its derivatives (i.e. GO and reduced GO [110]) have been adopted as electrodes for the construction of electrochemical biosensors due to their reversible electrical oxidation and reduction, high specific surface area, and outstanding conductivity [111]. Based on the high synergistic effect between GO and metal oxide particles, the unique electronic properties of GO can be tailored by coating it with metal NPs [112,113]. Devi and coworkers developed a glassy carbon electrode that was modified using a AuNPs/nickel ferrite decorated GO-chitosan nanocomposite (Au/$NiFe_2O_4$@GO-Ch) for Aβ42 quantification [112]. As shown in Fig. 4A, the working electrode was modified by coating Au/$NiFe_2O_4$@GO-Ch composite, followed by immobilization of Aβ antibody. After a blocking step with BSA, the electrode was incubated with different concentrations of Aβ42 peptides, and the electrochemical response was measured by electrochemical impedance spectroscopy, differential pulse voltammetry, and cyclic voltammetry. The immunosensor exhibited superior sensitivity for the clinical detection of Aβ42 in the CSF with low LOD of 3.0 pg/mL, and a wide linear range of 1 pg/mL to 1 ng/mL was obtained after a 30 min incubation of Aβ42 with the immunosensor, suggesting synergistic electrocatalysis between Au/$NiFe_2O_4$ NPs and Ch-modified GO in this

sensing model. To improve the specific surface area as well as sensor's selectivity and stability, several sensors were fabricated by assembling GO and rGOGO and rGO into multilayered nanocomposite. Sethi's groups reported for the first time the combination of GO and rGO into one sensing system, and this system exhibited rapid and significantly higher sensitivity for the specific detection of Aβ40 in both spiked human and mice plasma samples with low LOD of 2.398 pM that could be detected after a 60 min incubation with the sensor [114]. These results represented a significantly improved performance compared to that of GO SPE alone [115]. Recently, the functionalization of graphene using a highly active Co_9S_8 compound was designed for use in another electrochemical sensor. To facilitate the electron transferability of the sensing platform and to enhance the electrocatalytic activity toward the H_2O_2 redox reaction, palladium nanoparticles were doped onto a graphene surface, resulting in G/Co_9S_8-Pd nanocomposites that exhibited a remarkable sensitivity for Aβ detection in artificial CSF samples, where the LOD was 41.4 fg/mL [113]. The use of carbon nanomaterials in such sensing platforms allows for considerable advances in rapidity, selectivity, and sensitivity, thus highlighting their potential applications for use in early diagnosis AD.

Despite the superior features of carbon nanomaterials, only a few biosensing systems have been proposed for the quantification of Tau-employing carbon nanomaterial-based methods [116–119]. Notably, the co-assembly of MWCNT and rGO as a hybrid has been exploited, resulting in a nanocomposite that exhibits much higher electrical conductivity and a larger specific active area. Li and colleagues introduced an electrochemical sensor based on biocompatible chitosan-decorated MWCNT-rGO (MWCNT-rGO/CS) for the detection of Tau441 in the serum of patients with AD [116]. The procedure used for this sensor fabrication is illustrated in Fig. 4B. The nanocomposite MWCNT-rGO/CS was bonded onto the electrode surface, followed by the immobilization of a specific antibody against Tau441. The combination of MWCNT-rGO and chitosan provides not only more specific surface area and improved dispersibility of the nanocomposite but allows stable binding of antibodies on the modified electrode. To achieve further signal amplification, AuNPs were used to modify the Tau441 protein. This multi-amplified sensing system exhibited a satisfactory sensitivity with LOD as low as 0.46 fM and was used for successful application in the human serum. In another study, for the determination of Tau441, rGO-Au nanocomposites were integrated, and the anchoring compound 11- mercaptoundecanoic acid (11-MUA) was used to facilitate strong covalent interaction between rGO and Au. Subsequently, anti-Tau antibody was immobilized onto the nanocomposite, allowing a specific detection of Tau441. As a result, a biosensor with a highly repeatable and reproducible capacity for Tau441 analysis in both CSF and serum was achieved [117].

Currently, almost all electrochemical sensors for AD marker detection were designed to target a single analyte. However, to increase the diagnostic reliability and accuracy, a multiplex detection approach is more desirable. Recently, a CNT-based multiple detection platform for the simultaneous detection of Aβ42, Aβ40, t-Tau, and p-Tau181 was developed [120] (Fig. 4 C). For the sensor fabrication, first, SWCNTs were repeatedly compressed and rebounded for 10 cycles to form densely aligned CNT monolayer films. The CNT films were then integrated with metallic source and drain electrodes and modified with specific antibodies against Aβ42, Aβ40, t-Tau, or p-Tau181. This densely packed multiplex sensor showed great performance with accurate and reliable detection of AD biomarkers at the lowest-recorded LOD in the femtomolar range (2.13 to 2.72 fM). The clinical validity of this sensor was also examined through measuring the analytes in pooled blood plasma. The results showed a change in array's resistance which is correlated to the change of biomarker levels within a clinically related range, suggesting the applicability of the sensor array in clinical detection of AD multiple biomarkers.

It must be noted that carbon material, once functionalized, may undergo an extensive reduction in an active surface, and this can lead to an increased background current that can reduce the LOD [121]. Furthermore, a low dissolving rate and a high accumulation tendency are the other drawbacks of carbon-based surfaces [121] during clinical assays. To overcome these limitations, other non-carbon materials have been adopted for the detection of Aβ, including light-switch molecule-integrated bipolar electrode electrochemiluminescence materials [122], conductive hydrogel polymer fibers [123], electropolymerized functionalization 173 materials [124], metal nanoparticles (NPs) [125–138], natural compound-metal NPs [139], and self-assembly monolayer-based [140] electrode modifications. Based on the excellent properties of peroxidase mimics, such as high stability against pH and temperature compared to those properties in natural enzymes, a luminophore/coreactant/co-reaction accelerator ternary electrochemiluminescence system has been developed [130]. As shown in Fig. 5A, a prepared Fe_3O_4@PPy-Au composite was coated onto glassy carbon electrodes (GCE) followed by incubation with an Aβ42 capture antibody. Another nanocomposite containing a functionalized metal-organic framework (Co-MOFs/ABEI-Au) that acts as a co-reaction accelerator for the catalysis of H_2O_2 decomposition was decorated with an $A\beta_{42}$ detection antibody. Aβ42 concentrations were measured using an MPI-F flow-injection chemiluminescence detector based on a sandwiched immunoreaction. After a 40 min incubation of Aβ42 in the sensor, an ultralow LOD of 3 fg/mL was obtained with a wide range of 10 fg/mL-100 ng/mL. Another metal NPs, $MnCO_3$ with excellent electrochemiluminescence (ECL) property has also been exploited [129]. $MnCO_3$ nanospheres were used as a novel luminophore that exhibited high biocompatibility, high stability, and low toxicity. Polydimethyldiallylammonium chloride functionalized $MnCO_3$ ($MnCO_3$/PDDA) was combined with AuNPs to form a $MnCO_3$/PDDA/Au composite. To improve antibody stability and sensitivity, an HC-7 heptapeptide was used. The biosensing platform was successfully applied for the analysis of Aβ in human CSF samples with very low LOD (19.95 fg/mL), and the detected signal was

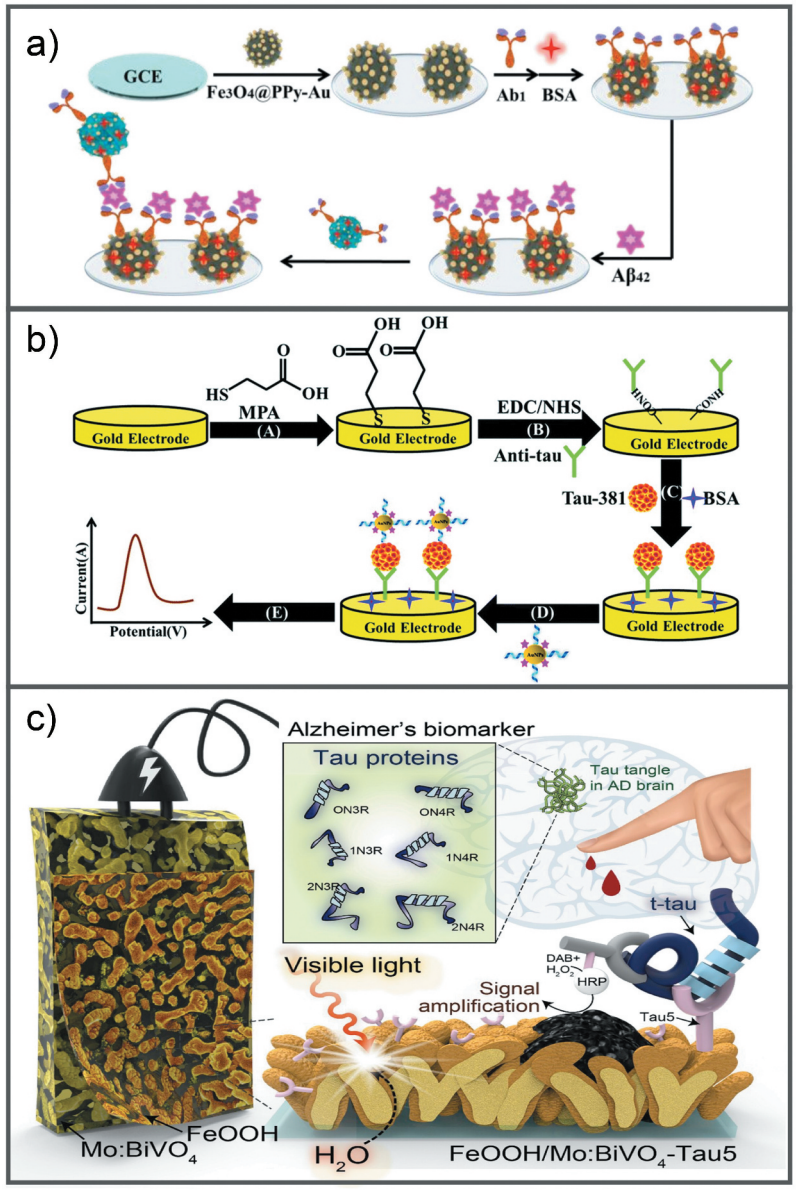

Figure 5. The preparation process of Cobalt-based metal-organic frameworks for Aβ42 detection. Adapted with permission from Ref. [131]. B) The preparation procedure for Tau381 detection using the aptamer-antibody sandwich assay. Adapted with permission from Ref. [144]. C) The fabrication process of the water oxidation-coupled, FeOOH/Mo:BiVO4-based photoelectrochemical sensing system used for Tau detection. Adapted with permission from Ref. [142].

almost unchanged after more than 30 min of incubation with Aβ in the sensor. Given the greatly improved performances of the sensing devices mentioned above, non-carbonaceous materials are highly recommended for the development of electrochemical platforms that can specifically and accurately detect Aβ levels during AD progression.

The utilization of such metal NPs for sensor fabrication also showed superior sensitivity toward the detection of low-abundance Tau proteins [141–146]. The excellent signal amplification properties of AuNPs have been successfully employed in an aptamer-antibody sandwich-based electrochemical sensing array for the detection of Tau381 in human serum (Fig. 5B) [143]. The gold electrode was first modified with mercaptopropionic acid (MPA) to improve the stability of the antibody. The anti-tau antibody-modified electrode was then allowed to immunoreact with Tau381 protein, followed by an affinity interaction with aptamer-Au-chitosan-bioconjugate. The level of Tau381 in the serum was assessed by differential pulse voltammetry. The proposed biosensor exhibited rapid, simple, and highly sensitive performance with LOD of 0.42 pM for the detection of Tau381 in biological samples, and the response was stable for up to 45 min. Most notably, a novel platform that allowed for extremely sensitive detection of total Tau in the blood plasma of patients with AD was introduced [141]. As shown in Fig. 5 C, the detection platform was fabricated using a ternary oxide-based semiconducting material-$BiVO_4$

photoelectrode that exhibits substantial absorption of visible light and good performance regarding water oxidation. To accelerate electron-hole separation in the $BiVO_4$ electrode, the hexavalent Mo ion was doped onto the $BiVO_4$ surface (Mo:$BiVO_4$). This surface was then decorated with an electrocatalyst FeOOH layer to facilitate the water oxidation reaction (FeOOH/Mo:$BiVO_4$). To enhance the degree of photocurrent signal, a sandwich-type sensing assay consisting of a detection antibody (Tau5 antibody conjugated with FeOOH/Mo:$BiVO_4$ nanocomposite) and an HRP-labeled capture antibody (Tau46 antibody) was utilized. In the presence of t-Tau, the photocurrent signal was amplified via peroxidase-catalyzed oxidation of 3,3′- diaminobenzidine. As a result, a highly accurate and ultrasensitive detection of t-Tau was achieved after a 60 min incubation of t-Tau in the sensor with LOD down to femtomolar concentrations (1.59 fM). This system represents a new, accurate, and sensitive approach for the quantification of low-abundance Tau protein in the samples of patients with AD.

Polymer is another material currently used for electrode modification that offers high biocompatibility [147] and high mechanical properties [148]. Several polymer-modified electrochemical sensors have been fabricated for the detection of AD biomarkers. Typically, the electroactive polymer poly (1-(2-carboxyethyl)pyrrole) (PPyCOOH) was used in couple with cellular prion protein $PrP_{95–110}$ peptide for the detection of AβO [149]. The sensor was fabricated by integrating PPyCOOH on the surface of gold electrode, followed by the immobilization of $PrP_{95–110}$ as a biorecognition element. The binding of $PrP_{95–110}$ peptide and AβO leads to the change in the EIS curve, which is proportional to the concentration of AβO. This sensing platform allowed a broad range of detection (10^{-7} to 10 nM) with LOD of 10^{-7} nM. The sensor also exhibited high selectivity toward AβO detection in compared to Aβ monomer and fibril. Other types of polymer materials were exploited for the development of electrochemical sensors that showed sensitive detection of different isoforms of Aβ. These include the utilization of electropolymerized Poly(3,4-ethylenedioxythiophene) (PEDOT) linked with conductive silk fibroin-based immunoparticles (CSIPs) [123] or co-polymer of polytyramine/3-(4-hydroxyphenyl) propionic acid (POPA)-attached biotin-$PrP_{95–110}$ peptide [124]. These polymer-modified sensors exhibited ultrasensitive measurement of either AβO in conditioned medium [124] or Aβ42/Aβ40 in AD mouse brain *in vivo* model [123]. The application of these new materials with great properties for electrochemical sensor fabrication has offered numerous advantages toward the accurate, sensitive and selective detection of core biomarkers during early onset of AD in simple, rapid and low-cost procedures.

5. Clinical applicability of nanobiosensors for Aβ and Tau in accurate AD diagnosis

The changed levels of CSF Aβ and Tau have been used as clinical criteria for AD diagnosis, providing accurate diagnosis of AD. Accumulating data have demonstrated the clinical value of these markers in the early diagnosis of AD progression. While Aβ42 has been well known as a key molecule in AD hallmarks, the concentration ratio between Aβ42/Aβ40 [150] and p-Tau181/Aβ42 [151] have proven a more accuracy in the identification of AD compared to single ones. The ratio of Aβ42/Aβ40 has shown ≥80% accuracy for the discrimination of AD with other neurodegenerative dementia syndromes [152,153]. Similarly, the combination of p-Tau181-to-Aβ42 ratio has aided correct differentiation of AD from non-AD dementia with 86% accuracy [151] and AD from healthy control (92%) accuracy [154]. Therefore, multiple-marker detection is currently desirable to improve the diagnosis accuracy of AD at the early disease stage. To increase the clinical validity of AD diagnosis, several strategies have been employed for developing simultaneous detection platforms of multiple biomarkers [70,73,82,90,93,120,155]. These advancements include the immobilization of monoclonal antibodies on different metal nanoparticle-based nanocomposites or the conjugation of hybrid antibody/aptamer on metal NPs, which provide superior performance compared to conventional detection methods, thereby allowing detecting Aβ and Tau simultaneously and accurately. Even though most of these sensing platforms are limited to spike sample or artificial CSF detection only, these emerging biosensors promise to ensure clinically standardized diagnostic assessment for AD. Among the currently developed biosensors, a couple of sensing platforms have been successfully applied for clinical use. Demeritte and colleagues have developed a multifunctional GO-hybridized plasmonic-magnetic nanoplatform capable of selectively capturing more than 98% Aβ and Tau in whole blood samples, indicating its reliability in clinical diagnosis [93]. Other turn-on fluorophore sensors were fabricated using specific antibodies coupled magnetic NPs, showing their practical utility in different body fluids, including serum, CSF, urine, saliva [70,73]. These sensors could precisely and simultaneously detect Aβ42 and Tau441 in the human clinical samples, showing the detection sensitivity comparable to that obtained by ELISA [73] and allowing a clear distinguishment of AD patients from young control and elderly control groups [70]. Notably, a densely aligned CNT-based electrochemical sensing array for multiplex detection of Aβ42, Aβ40, t-Tau, p-Tau181 was recently developed [120]. The distinct property of this sensing system is the immobilization of four different bioelements into a single closely aligned CNT that allowed the simultaneous detection of AD core proteins at ultra-low levels. The clinical applicability of this sensing array was validated on AD patients and healthy controls. Importantly, when using a single biomarker, the sensing system exhibited a range of sensitivity and selectivity from 70.0% to 88.3%, which is not sufficient for the differentiation between AD patients and healthy controls. Whereas, by simultaneously detecting multiple biomarkers, the sensing array showed excellent clinical applicability as it successfully identifies the AD patients with an average accuracy of 90.2%. Conclusively, the combination of core biomarkers

Aβ and Tau provides more accurate and sensitive detection of AD, thereby holding potential clinical applicability for the diagnosis of early onset AD.

6. Conclusion

This review provides an overview of the cooperation between Aβ and Tau pathological pathways and summarizes the most recent nanomaterial-based optical and electrochemical biosensors used for the detection of these validated biomarkers for the early potential diagnosis of AD. Aβ and Tau pathological pathways suggest that these proteins are beneficial biomarkers for use in AD diagnosis. Advanced optical and electrochemical biosensors that incorporate nanostructures exhibit outstanding advantages in the early diagnosis of AD, thus making them preferable biosensors compared to conventional approaches. These biosensing platforms may become a standard for the clinical diagnosis of AD with high potential for global applicability and will open doors for efficient AD control to eliminate the economic and social burdens of AD.

7. Expert opinion

To date, the global increase in life expectancy has also increased the risk of disease, dementia, disability, and age-related diseases. In particular, AD is a growing epidemic that represents the primary cause of dementia worldwide and has resulted in an astronomically devastating socioeconomic burden. Unfortunately, the diagnosis of AD routinely occurs after the appearance of irreversible damage to the brain. Therefore, the development of sensitive diagnostic tools is highly desirable to achieve an early and accurate diagnosis of AD to prevent irreversible and uncontrollable consequences. AD biomarkers, particularly Aβ and Tau that possess important early diagnostic value, exhibit promising potential in routine clinical diagnostics for the early diagnosis of AD. Advanced nanomaterial-based biosensors for AD have demonstrated great advantages such as ultralow detection sensitivity, rapid detection time, and the elimination of expensive instruments compared to conventional approaches. A number of optical and electrochemical biosensors have been successfully developed to quantify Aβ and Tau at ultralow femtomolar or picomolar concentrations, and this is thought to be sufficient for accurate monitoring of AD before the manifestation of clinical symptoms. Nanomaterial-based optical biosensors, including colorimetric, fluorescent, SPR, and SERS-based sensors, possess advantages in that they provide label-free and real-time sensing platforms with ultrasensitivity in the early diagnosis of AD. On the other hand, the advantages of the fabrication of electrochemical biosensors using nanostructures include robustness, easy miniaturization with excellent sensitivity and the need for only small analyte volumes. Although the levels of Aβ and Tau biomarkers in CSF are estimated with higher accuracy and can be achieved earlier with regard to brain tissue damages compared to those in plasma, an invasive procedure of lumbar puncture is required to obtain CSF samples for biomarker detection. Hence, recently developed nanomaterial-based optical and electrochemical biosensors possess the potential to provide adequate sensitive diagnostic tools for the detection of Aβ and Tau biomarkers in plasma with less invasive sampling methods. Moreover, a significantly higher concentration of Aβ and Tau was observed in saliva [156], tear [157], nasal secretions [158,159] of AD patients than that of normal people. These biofluids could supply as good noninvasive sources to detect these AD biomarkers, leading to an efficient noninvasive approach to monitor AD. It is also worth noting that most biosensors have been generated to detect a single biomarker at a given time, resulting in insufficient criteria to achieve more definitive conclusions. In 2018, the National Institute of Aging and Alzheimer's Association emphasized the AD's diagnostic criteria for grouped biomarkers (β amyloid deposition, pathologic Tau, and neurodegeneration) as secondary diagnostic tool. The measurement of Tau alone is not sufficient for AD confirmation because Tau is also an indicator for other neurological diseases. The combination of Aβ, T-Tau, and P-Tau is recommended for the accurate diagnosis of syndromal cognitive staging [5]. We believe that in the future, nanosensor platforms capable of detecting multiple AD biomarkers will be intensely developed to increase diagnostic validity and achieve a more reliable clinical conclusion. It is also expected that more efficient sensor devices integrating various nanomaterials for simultaneous recognition of multiple AD biomarkers will emerge in the not-so-distant future to achieve higher clinical sensitivity and specificity. Nanomaterial-associated diagnostic platforms that can rapidly detect multiple biomarkers of AD and that possess excellent clinical sensitivity and specificity will ultimately allow for the commercialization of biosensors for point-of-care diagnosis of AD. Additionally, the medical internet-aided diagnosis that incorporates the use of reusable wearable sensors for AD biomarkers may ultimately improve the accurate clinical diagnosis of AD by facilitating remote patient monitoring. These nanobiosensor strategies will drive the next wave of innovative diagnosis of AD to provide significant contributions to primary Alzheimer's care intervention.

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Declaration of interest

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