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A review of recent advancement in laboratory diagnostic testing for neurodegenerative diseases

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Abstract

Neurodegenerative diseases (NDDs), such as Alzheimer's disease (AD), Parkinson's disease (PD), and prion diseases, are characterized by the accumulation of misfolded proteins and are projected to become the second leading cause of death worldwide by 2040. The development of sensitive and efficient detection technologies for these disorders is crucial for early diagnosis and understanding the underlying pathological mechanisms. This review critically evaluates recent progress in the use of electrochemical and optical biosensors for the detection of NDDs. Optical biosensors, such as surface plasmon resonance imaging (SPRi), have been employed to detect AD biomarkers, including amyloid-β (Aβ), tau, and apolipoprotein E (ApoE), with high sensitivity. Similarly, quantum dot (QD)-based biosensors have been developed for detecting PD biomarkers, such as mitochondrial complex I abnormalities and dopamine levels. Electrochemical biosensors have also been utilized for the detection of AD and PD biomarkers, employing various strategies such as the use of specific antibodies, aptamers, and nanomaterials. For prion diseases, both optical and electrochemical biosensors have been developed, utilizing techniques such as SPR, chemiluminescence, and field-effect transistors (FETs). Despite these advancements,

challenges remain in integrating multiple analytical technologies onto a single platform, transitioning from the development phase to commercialization, and exploring new nanomaterials and biomimetic surfaces to further enhance the sensitivity and reliability of NDD detection. Addressing these challenges will be essential for the continued evolution of biosensors and their translation into point-of-care diagnostic tools for NDDs.
Keywords: laboratory diagnostic testing, neurodegenerative disease,

1. Introduction

Neurodegenerative diseases (NDDs), including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and prion diseases such as Creutzfeldt-Jakob disease (CJD) and Bovine Spongiform Encephalopathy (BSE), are classified as 'protein mis-folding disorders.' These diseases share a common pathological feature: the accumulation of aggregation-prone proteins, such as Amyloid-β (Aβ) in AD, α-synuclein in PD, huntingtin in HD, and Prion protein (PrP) in prion diseases. These misfolded proteins have been implicated in the etiology of these conditions. According to World Health Organization (WHO) projections, by 2040, NDDs will surpass cancer to become the second leading cause of death worldwide, with an estimated economic burden of $2 trillion (USD) by 2030. The non-human costs of these diseases are also considerable; for instance, prion diseases like BSE, also known as Mad Cow disease, can devastate livestock populations, leading to billions of dollars in trade losses following an outbreak. Consequently, the development of technologies capable of detecting these 'protein mis-folding disorders' in an efficient and sensitive manner is essential, enabling early diagnosis and advancing our understanding of the underlying pathological mechanisms crucial for combating these diseases.

Numerous analytical technologies have been developed to detect and elucidate the mechanisms underlying these diseases, with the hope of fostering effective therapeutic interventions. Recent advances in material science, including carbon nanotubes (CNTs), gold nanoparticles (AuNPs), and quantum dots (QDs), have led to the emergence of next-generation biosensors. These biosensors have been creatively applied to address the challenges posed by NDDs. This review aims to critically evaluate recent progress in the use of electrochemical and optical biosensors for the detection of NDDs.

1.1. Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system marked by progressive memory loss and cognitive decline. Currently, 46 million individuals are living with AD globally, and this number is expected to triple by 2050. The pathological features of AD include extracellular deposits of dense-core amyloid plaques, which exhibit a positive stain for thioflavin-S and Congo Red, neurofibrillary tangles (NFTs), cerebral amyloid angiopathy (CAA), and neuronal and synaptic loss in the cortical areas (Serrano-Pozo et al., 2011). The amyloid plaques are formed due to the abnormal accumulation and deposition of Aβ peptides, specifically Aβ40 (40 amino acids) and Aβ42 (42 amino acids), which are normally produced as by-products from the sequential cleavage of the Amyloid Precursor Protein (APP) by β- and γ-secretases. Aβ is an amphipathic peptide, typically consisting of 38 to 43 amino acids, with the most prevalent forms being Aβ40 and Aβ42. Despite amyloid plaques composed of aggregated Aβ being recognized as the hallmark of AD pathology, substantial evidence indicates that the oligomeric intermediates formed during Aβ aggregation may be the true toxic species. Furthermore, studies have suggested that the inheritance of the E693Δ mutation (Osaka mutation) in Aβ, which impedes the formation of insoluble amyloid fibrils while promoting the production of soluble Aβ oligomers, results in a condition resembling AD (Nishitsuji et al., 2009). These findings have directed research efforts toward focusing on the toxic Aβ oligomers as a likely cause of AD.

Initially, it was hypothesized that amyloid plaques were directly responsible for the onset and progression of AD. However, more recent studies have challenged this oversimplified view of AD pathology. For instance, a landmark study by Giannakopoulos et al. in 2003 demonstrated that neurofibrillary tangles (NFTs) and neuronal loss, but not amyloid plaque burden, were more reliable predictors of cognitive decline in AD patients. The role of Tau protein in the formation of NFTs in AD has been extensively studied (Anand et al., 2014; Kopeikina et al., 2012). In healthy neurons, Tau binds to microtubules and helps maintain cellular structure and function. In AD, however, Tau becomes hyperphosphorylated, causing it to detach from the microtubules and aggregate into insoluble NFTs and paired helical filaments (PHFs), disrupting cellular functions and leading to neuronal death. Tau aggregation progresses through the formation of various soluble oligomers, which mature and further aggregate into insoluble PHFs and NFTs. It is believed that the soluble tau oligomers are neurotoxic (Lasagna-Reeves et al., 2010) while the insoluble mature PHFs and NFTs may play a protective role by acting as nucleation sites for the removal of soluble oligomers. Despite this, soluble tau oligomers remain important targets for drug development. However, targeting tau pathology in AD continues to be a significant challenge.

There is substantial evidence supporting the involvement of apolipoprotein (Apo) as a key risk factor in Alzheimer's disease (AD). ApoE is a polymorphic lipid-binding protein that exists in three common allele variants: ApoE2, ApoE3, and ApoE4. The ApoE4 allele is the most significant and only confirmed genetic risk factor for the development of late-onset AD. Heterozygous carriers of the ApoE4 gene face a threefold increased risk, while homozygous individuals are at a twelvefold increased risk for the disease. Among the three isoforms, ApoE3 is the most prevalent, with an incidence of 78%, followed by ApoE4 at 15%, and ApoE2 at 7%. The high-risk ApoE4 isoform has been linked to the amyloidogenic pathway in AD. The structural difference between these isoforms arises from variations at positions 112 and 158 in the N-terminal domain. ApoE3 contains Cys/Arg, ApoE4 has Arg/Arg, and ApoE2 features Cys/Cys. These changes in the primary structure are known to decrease the stability of the N-terminal helical bundle, facilitating ionic interactions between the N- and C-terminal domains. This interaction has been associated with the preference of ApoE4 to bind very low-density lipoproteins (VLDL) rather than high-density lipoproteins (HDL). Several in vitro studies have shown that lipid-free ApoE4 binds Aβ with higher affinity than ApoE3, promoting fibril formation. In contrast, ApoE3's interaction with smaller oligomeric Aβ species may facilitate their exchange onto lipoprotein particles at the blood-brain barrier. It is hypothesized that ApoE2 and ApoE3 are not high-risk isoforms because of the formation of disulfide bridges between the Cys residues during dimerization. The presence of Arg at position 112 in ApoE4 (instead of Cys in ApoE2 and ApoE3) leads to electrostatic docking of the C-terminal domain with the N-terminal helical bundle, reducing C-terminal availability and thus explaining ApoE4's lower binding affinity for toxic oligomeric Aβ species. Although ApoE4 has shown potential as a promising biomarker, its reliability, particularly in the early diagnosis and monitoring of AD, has been questioned. For a more detailed analysis of AD biomarkers, refer to the review by Hampel et al. (Hampel et al., 2010). It is increasingly recognized that AD is a multifactorial disease, caused by the complex interaction of multiple pathogenic factors, including APP/Aβ, ApoE4, tau, aging, and various co-morbidities (Huang & Mucke, 2012).

1.2. Parkinson's disease

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra (SN) of the brain, leading to clinical manifestations such as bradykinesia, rigidity, tremors, and postural instability. Pathologically,

PD is marked by a 50–70% loss of neurons in the SN region, along with the presence of neuronal inclusions composed of α-synuclein protein, termed 'Lewy bodies' in the neuronal cell body and 'Lewy neurites' in the neurites (Dickson, 2012). Growing evidence suggests that the dysregulated folding and aggregation of α-synuclein in Lewy bodies play a pivotal role in the pathogenesis of PD. Oligomers of α-synuclein have also been proposed as the toxic species responsible for neuronal death during the early stages of PD. There is strong evidence indicating that α-synuclein oligomer-mediated neurotoxicity results from morphological changes in α-synuclein induced by the presence of metal ions. While PD is predominantly considered to be an idiopathic (sporadic) disease, there is significant evidence pointing to the involvement of several genetic risk factors. Mutations in the Leucine Rich Repeat Kinase 2 gene (LRRK2/PARK8) are the most common cause of the familial form of PD, occurring in 5–7% of patients with a family history of the disease. Additional potential risk factors for PD include mutations in genes such as Parkin, DJ-1, SNCA (the α-synuclein gene), and PARK4, along with abnormalities in mitochondrial complex I, dopamine (DA) regulation, and the microtubule-associated protein Tau gene (MAPT) (Shulman et al., 2011).

1.3. Prion Diseases

Neurodegenerative diseases such as Creutzfeldt-Jakob disease (CJD), Bovine Spongiform Encephalopathy (BSE), Kuru, Gerstmann-Straussler-Scheinker syndrome (GSS), and Fatal Familial Insomnia (FFI) are categorized as prion diseases, which belong to a group of disorders known as Transmissible Spongiform Encephalopathies (TSEs). TSEs are progressive, transmissible, and ultimately fatal neurodegenerative disorders caused by the misfolding and aggregation of a host-encoded protein called Prion Protein (PrP). PrP is believed to exist in two distinct conformational states: the normal, non-pathogenic form (PrPC) and the pathogenic, misfolded form (PrPSC), which serves as the infectious agent that triggers the conversion and polymerization of PrPC into the harmful PrPSC form in healthy cells. Although TSEs are primarily sporadic, there are also iatrogenic (transmitted) and familial forms of the disease that contribute to a significant proportion of cases. Various risk factors for TSEs have been identified, including mutations or variants in the PrP codon 129, exposure to brain tissue or extracts contaminated with the TSE agent, infection by exogenous viral agents, the presence of additional genetic factors, age of onset, and environmental or dietary factors.

2. Optical Biosensors in NDD Detection

2.1. AD Detection

Biosensors are highly effective tools for detecting AD biomarkers in body fluids, such as cerebrospinal fluid (CSF) and blood. Currently, the main focus in the development of biosensor technology is to enhance detection sensitivity. One such method, Surface Plasmon Resonance imaging (SPRi), has been employed to detect and measure Aβ42 levels in serum (Zhao et al., 2015). This technique involves the fabrication of Alzheimer's disease peptoids (ADP3) on gold-coated glass chips. Peptoids, which are synthetic N-substituted oligoglycines, are used as antigen surrogates to isolate target antibodies, such as those specific to Aβ42, from body fluids. ADP3, when used at high concentrations, successfully detected Aβ42 levels in serum, even at concentrations as low as 89 nM. The use of serum sampling for AD detection shows great promise as an efficient diagnostic strategy, as it eliminates the need for the invasive and painful procedure required for CSF collection. In a study by Lee et al., a Waveguide-coupled bimetallic (WcBiM) SPR chip in the intensity measurement mode was used to measure Aβ42 levels in the picogram per milliliter (pg/mL) range. This high sensitivity is particularly important, as physiological Aβ42 levels in the CSF of AD patients are reported to be below 500 pg/mL. Other research groups have also utilized the SPR technique to detect AD biomarkers, albeit with variations in approach. For instance, using SPR, Xia et al. were able to detect both Aβ40 and Aβ42 in the CSF of AD patients by employing capture antibodies specific to each peptide in

separate fluidic channels (Xia et al., 2010). The signal was further amplified using a streptavidin-conjugated antibody that selectively bound to the common N-terminus of the Aβ peptides that had already been captured, achieving a detection limit as low as 20 pM. These methods offer significant advantages over currently available optical detection techniques for Aβ, such as enzyme-linked immunosorbent assay (ELISA), which typically detects Aβ in a range of 5–250 pg/mL.

SPR has also been utilized to detect AD biomarkers other than Aβ42 peptides. For instance, Sciacca and colleagues employed a silver-coated emission-based fiber SPR platform to detect apolipoprotein E (apoE), a protein of approximately 39 kDa that has been implicated in Alzheimer's disease (Sciacca et al., 2013). Additionally, PCR-coupled DNA biosensors that use techniques such as SPR and quartz crystal microbalance have been reported for detecting the apoE gene. Vestergaard et al. introduced a localized SPR (LSPR)-based immunochip for detecting tau, a 50–65 kDa protein biomarker associated with AD. LSPR is an optical phenomenon that occurs when light interacts with conductive nanoparticles (NPs) smaller than the wavelength of the incident light. The incident light's electric field excites the conduction band electrons, generating coherent localized plasmon oscillations with a resonant frequency that is influenced by the composition, size, geometry, dielectric environment, and separation distance of the NPs. Using the LSPR technique, the researchers successfully detected tau proteins using anti-tau antibodies with high specificity at concentrations as low as 10 pg/mL, even in a complex protein environment. The detection limit achieved was superior to the cut-off tau concentration in CSF (195 pg/mL), which is used to differentiate AD patients from non-AD individuals. These methods provide significant advantages compared to existing tau detection techniques, such as ELISA, which typically have detection ranges from 30 to 2,000 pg/mL.

In addition, innovative optical biosensors employing novel surface modifications have been developed (Gagni et al., 2013). For example, Gagni and colleagues coated silicon chips with a ter-copolymer made of dimethylacrylamide, 3-(trimethoxysilyl) propyl methacrylate, and N-Acryloyloxy succinimide, resulting in a silicon micro-array capable of enhancing fluorescence signals at the surface (Gagni et al., 2013). This micro-array was utilized as a high-sensitivity immunoassay platform, and testing with an artificial CSF sample revealed an Aβ42 detection sensitivity of 73 pg/mL. Similarly, Ammar et al. silanized a silicon wafer with either 7-octenyltrichlorosilane or carboxylated alkyltrichlorosilane to develop bio-receptive surfaces for use in immunoassay platforms aimed at detecting AD biomarkers such as Aβ42 through fluorescence techniques (Ammar et al., 2013). Morales et al. demonstrated that signal enhancement could be achieved by using Quantum Dots (QDs) in immunocomplex microarrays instead of traditional fluorophores like Alexa or conventional ELISA. Their findings indicated that QDs exhibited greater sensitivity than Alexa and ELISA, especially at lower excitation wavelengths.

2.2. PD Detection

Optical biosensors have also shown promise for the early, rapid, and sensitive detection of Parkinson’s disease (PD) biomarkers in human body fluids, such as CSF and blood. Ma et al. developed a novel QD-based biosensor for detecting mitochondrial complex I abnormalities associated with PD (Ma et al., 2013). In this method, ubiquinone-terminated disulfide ligands (QnNS), prepared through a 'Click Reaction,' were self-assembled onto the surface of 550 nm emitting Core-shell CdSe/ZnS QDs. The QD bio-conjugate (QnNS-QDs) exhibited fluorescence enhancement when in proximity to a properly functioning mitochondrial complex I. Conversely, any damage to complex I resulted in a decrease in fluorescence. Using human

neuronal cells (SH-SY5Y), the authors demonstrated that this biosensor had the potential for intracellular detection of PD-related abnormalities.

QDs have also been successfully utilized for the detection of PD biomarkers, such as dopamine (DA). Ankireddy et al. reported the development of an indium phosphide/zinc sulfide (InP/ZnS)-based QD biosensor for detecting DA levels in the presence of ascorbic acid (AA) (Ankireddy & Kim, 2015). The surface of the QDs was modified with L-cysteine through a coupling reaction, and DA was detected by fluorescence quenching of cysteine-capped InP/ZnS QDs in the presence of AA. Similarly, Yildirim et al. presented an optical method for detecting DA based on the intrinsic fluorescence properties of polydopamine nanoparticles, which are synthesized by oxidizing DA. This simple assay demonstrated high selectivity for DA, with a reported detection limit of 40 nM. Recent studies have also explored the possibility of detecting multiple biomarkers to enhance the reliability of PD detection. For instance, Kruse and colleagues developed an electrochemiluminescence (ECL)-based multiplex assay capable of simultaneously quantifying multiple biomarkers, including α-synuclein, in the CSF of PD patients. Moreover, emerging research indicates that combined detection of α-synuclein and other biomarkers in CSF reveals PD-specific patterns, thus supporting the development of multiplex assay strategies targeting multiple PD biomarkers.

2.3. Prion Disease Detection

A variety of optical biosensors have been developed to facilitate the sensitive and reliable detection of Prion Protein (PrP), the infectious agent in Transmissible Spongiform Encephalopathies (TSEs). For example, Jiayu et al. reported the development of a rapid, label-free immunoassay for PrP detection using SPR. By immobilizing primary antibodies specific to PrPSC on SPR chips, the researchers were able to detect PrPSC at concentrations as low as 36 ng/mL in mouse serum, as well as in infected mouse brain homogenates. Similarly, Hossain et al. presented a high-throughput method for detecting PrP, based on a chemiluminescence reaction between a PrP-specific aptamer and 3,4,5-trimethoxyphenylglyoxal. They demonstrated that this aptamer-based biosensor was capable of detecting PrPSC at concentrations as low as 6.2 pM/spot, offering greater sensitivity than immunoassays using PrP-specific antibodies. The same research group also developed another chemiluminescence assay, utilizing a Peroxidase-labeled Dextran probe, which achieved an impressive detection limit of 20 fM for PrP. Other research teams have successfully employed gold nanoparticles (AuNPs) in PrP detection. Using dihydrophilic acid-modified gold nanoparticles (DHLA-AuNPs), Hei-Jie Zhang and colleagues created a biosensor for PrP detection. In the presence of PrP (recombinant prion protein was used in this study), the selective aggregation of DHLA-AuNPs was induced, resulting in a decrease in absorbance, which was detected as a color change from red to blue.

3. Electrochemical Biosensors in NDD Detection

3.1. AD Detection

A range of electrochemical biosensors have been developed for the detection of Alzheimer's disease (AD) biomarkers, such as Aβ40/Aβ42, tau, ApoE, and miRNA in cerebrospinal fluid (CSF) and blood. For instance, Yu et al. introduced a novel electrochemical biosensor for detecting Aβ levels, utilizing gelsolin, a secretory protein that binds specifically to Aβ40 and Aβ42 monomers (Yu et al., 2014). The biosensor was fabricated by immobilizing gelsolin onto screen-printed carbon electrodes (SPCEs), followed by the binding of thionine (Th) labels linked to gold nanoparticles (AuNPs), with the reduction of Th serving as the electrochemical readout. The researchers found that the combined use of multi-walled carbon nanotubes (MWCNTs) and AuNPs as substrates was more effective than using either alone. These findings are of particular significance since the ratio of Aβ42/Aβ40 in the CSF has been proposed as a reliable predictor of AD progression. In a similar vein, Liu et al. developed a

competitive assay-based electrochemical sensor to detect total Aβ, employing Aβ(1–16)-heme-AuNPs (Liu et al., 2013). Monoclonal antibodies (mAB) specific to the common N-terminus of Aβ were immobilized onto a gold electrode surface. The presence of native Aβ reduced the binding of the Aβ(1–16)-heme-AuNP complex onto the electrode, leading to a decrease in the reduction current. This method was tested using artificial CSF samples containing Aβ40 and Aβ42, with a reported detection limit of 10 pM. Electrochemical sensors have also been developed for the detection of tau protein, another key biomarker in AD. Tau protein in the AD brain is abnormally hyperphosphorylated and forms a major component of neurofibrillary tangles (NFTs), one of the pathological features of AD. For example, Esteves-Villanueva et al. created a protein-based electrochemical sensor for detecting tau. Tau protein was immobilized on a gold surface, and tau-tau interactions were monitored using electrochemical impedance spectroscopy (EIS).

ApoE is another important AD biomarker for which several biosensors have been developed (Cheng et al., 2014). To detect DNA hybridization related to specific point mutations in the APOE gene, Cheng et al. designed an Au nanoparticle-modified dual detection platform based on EIS and localized surface plasmon resonance (LSPR) (Cheng et al., 2014). Similarly, Marazza et al. reported a polymerase chain reaction (PCR)-coupled DNA electrochemical biosensor for detecting APOE genotypes by amplifying DNA extracted from blood. Hybridization reactions with single-stranded DNA oligonucleotides incorporated onto screen-printed electrodes (SPEs) were monitored using chronopotentiometric stripping analysis (PSA), with daunomycin as the indicator. Medina-Sanchez et al. introduced a novel electrochemical immunosensing system for detecting ApoE in plasma, employing electrochemical detection of quantum dots (QDs) via square wave anodic stripping voltammetry (SWASV). This approach integrated a magnetic pre-concentration step and an SPCE in a microfluidic system, achieving a detection limit close to 12.5 ng/mL, with a linear range from 10 ng/mL to 200 ng/mL. Other innovations in pre-concentration techniques have also been reported. For example, de la Escosura et al. employed porous magnetic microspheres to provide a more efficient surface for antibody immobilization. AD biomarkers, such as Aβ and ApoE, were then measured by the electrocatalytic activity of AuNPs. Recently, multi-channel screen-printed array electrodes have been developed to monitor nucleic acid hybridization of miRNA sequences associated with AD.

3.2. PD Detection

Yue and colleagues reported the development of an electrochemical biosensor system for detecting biomarkers of Parkinson's disease (PD) (Yue et al., 2014). Their work demonstrated that by fabricating vertically aligned ZnO nanowires on a 3D graphene foam structure, they were able to successfully detect dopamine (DA), a key PD biomarker, using differential pulse voltammetry (DPV), achieving a detection limit of 1 nM. Electrochemical biosensors for the detection of α-synuclein, another PD biomarker, have also been developed (Sierks et al., 2011). By immobilizing nanobodies, which are single-domain antigen-binding proteins specific to α-synuclein, researchers were able to detect α-synuclein at femtomolar concentrations through electrochemical impedance spectroscopy (EIS). Moreover, they demonstrated the ability to detect specific oligomeric forms of α-synuclein in CSF samples from PD patients using nanobodies designed for these oligomers. An et al. developed an Au-doped TiO2 nanotube-based photoelectrochemical immunosensor for detecting α-synuclein. In this study, Au-doped TiO2 nanotubes were deposited on both sides of a titanium foil. Primary antibodies (Ab1)

specific to α-synuclein were immobilized on one side, forming an immunocomplex with the antigen, followed by the attachment of an antibody (Ab2)-Au-glucose oxidase (GOx) bioconjugate. This resulted in a final sandwich structure consisting of TiO2-Au-Ab1-α-synuclein-Ab2-GOx. When placed in a detection solution containing glucose, the GOx in the immunosensor complex catalyzed the conversion of glucose to gluconic acid and hydrogen peroxide (H2O2). Irradiating the opposite side of the Ti foil (365 nm) with light enabled H2O2 to act as a sacrificial electron donor, depleting photogenerated holes on the electrode and enhancing charge separation efficiency. The authors also demonstrated that the double-sided Au-doped TiO2 nanotubes were more effective than the single-sided counterparts. Besides being an interesting application of photoelectrochemistry in disease detection, the sensor showed high specificity for α-synuclein, with a detection range from 50 pg/mL to 100 ng/mL and a detection limit of 34 pg/mL.

3.3. Prion Disease Detection

Wustoni et al. developed a field-effect transistor (FET)-based biosensor using thiamine as a probe for the specific detection of prion protein (PrP) in human serum. By employing a dual-ligand binding approach, they amplified the FET signal through the addition of Cu2+ ions, which bound to the prion protein-thiamine complex, inducing an additional positive charge on the gate surface of the FET. This approach allowed the successful detection of PrP at concentrations as low as 40 pM, exceeding the diagnostically relevant concentration of 2 nM. Some researchers have also utilized aptamer technology to design innovative biosensors for detecting PrP. Miodek et al. developed an electrochemical aptasensor based on polypyrrole modified with redox dendrimers. Specific aptamers for PrP were immobilized on a gold surface modified with conductive polypyrrole film, which was coupled with fourth-generation polyamidoamine dendrimers (PAMAM G4) and a ferrocenyl group as a redox marker. The interaction between the aptamer and PrP led to changes in the electrochemical signal of the ferrocenyl group, which was measured using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Using known concentrations of PrP spiked in serum samples, they validated their aptasensor and achieved a detection limit of 1 pM. Miodek and colleagues also developed another electrochemical aptasensor using multi-walled carbon nanotubes (MWCNTs) for the sensitive detection of PrP (Miodek et al., 2013). They immobilized PrP-specific DNA aptamers on MWCNTs modified with PAMAM G4 dendrimers, with a ferrocenyl redox marker incorporated between the dendrimer and aptamer layers. The proximity of the aptamer to PrP generated an electrochemical signal measured by CV, with a reported detection limit of 0.5 pM and a broad linear detection range from 1 pM to 10 μM. Hianik et al. also developed an electrochemical biosensor for the detection of cellular PrP using DNA aptamers and antibodies immobilized on carbon nanotubes (CNTs), reporting a detection limit ranging from 20 to 50 pM.

Conclusions and Future Directions

As the global population ages, the number of individuals affected by neurodegenerative diseases (NDDs) is expected to increase. This highlights the urgent need for sensitive, rapid, reliable, and cost-effective detection technologies for NDDs, based on emerging analytical methods. This review has presented an overview of recent advancements in the field, highlighting the progress made by biosensors utilizing optical and electrochemical techniques to address this pressing need. Despite these developments, challenges remain that must be addressed to sustain the field's growth. Three strategic development angles are particularly important for advancing the field. The first is the integration of analytical technologies onto a single platform. The multifactorial nature of NDDs presents an opportunity for more reliable detection through the simultaneous detection of multiple biomarkers. The development of biosensors capable of detecting various biomarkers of an NDD could improve detection

sensitivity while minimizing false positives. Additionally, integrating these various aspects onto a single platform would enhance the efficiency of high-throughput detection efforts. The second critical area is the transition from the development phase to the commercialization of biosensors for point-of-care diagnostics of NDDs. This is particularly crucial for biosensors with sensitivity and detection limits that exceed diagnostically and physiologically relevant levels. By advancing bench-top technologies toward product development, it will be possible to address other practical considerations, such as portability, cost, and fabrication techniques, thus facilitating the translation of these biosensors into point-of-care diagnostic tools. Lastly, exploring new advances in nanomaterials, nanofabrication technologies, and biomimetic surfaces will be essential for the continued evolution of biosensors that push the boundaries of NDD detection.

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