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Recent advances in biosensors for neurodegenerative disease detection

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ABSTRACT

Neurodegenerative diseases (NDDs) such as Alzheimer's disease (AD), Parkinson's disease (PD) and Prion diseases are known as *'protein misfolding disorders'* because aggregation prone proteins are postulated to be the underlying causative agents in these diseases. The economic impact of NDDs, including human and non-human costs, are quite staggering and represents a significant public health challenge for nations around the world. Technology development that facilitates early detection of NDDs, is therefore, the need of the hour. Various analytical technologies have been developed to address this challenge, in the hope of evolving effective therapeutic strategies against NDDs. Over the past decade, biosensors based on optical and electrochemical techniques have been at the forefront of this development, thanks to advances in material science such as carbon nanotubes (CNTs), gold nanoparticles (AuNPs), and quantum dots (QDs). In this review, we evaluate the most recent advances in optical and electrochemical biosensors for detection of NDDs.

Contents

1. Introduction

Neurodegenerative diseases (NDDs) such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Prion diseases such as Creutzfeldt-Jakob disease (CJD) Bovine Spongiform Encephalopathy (BSE) are termed as *'protein mis-folding disorders'*. The above mentioned diseases share a common feature, they are all characterized by aggregation prone proteins – *Amyloid-β* (Aβ) in AD, *α-synuclein* in PD, *huntingtin* in HD, *Prion protein (PrP)* in Prion diseases [1], which have been shown to contribute to the aetiology of these diseases. The World Health Organization (WHO) projections estimate that by 2040, NDDs will surpass cancer to become the second most leading cause of death worldwide [2], with an estimated economic impact of $2 trillion (USD) by 2030 [3]. Non-human costs of NDDs are also staggering. For example, Prion diseases such as BSE, commonly known as *Mad Cow disease*, can affect livestock and cause billions of dollars in trade losses in the event of a disease breakout [4]. Thus, it is imperative to develop technologies

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that allow for effective and sensitive detection of these *'protein misfolding disorders'*. This would allow for early detection and further understanding of pathological mechanisms that aid the endeavour of battling these diseases.

A variety of analytical technologies have been developed to detect and unravel the underlying disease mechanisms in the hope of developing effective therapeutic treatments. Recent advances in material science such as carbon nanotubes (CNTs), gold nanoparticles (AuNPs), and quantum dots (QDs) have given rise to the next generation of biosensors that have been effectively and ingeniously employed to address these challenges. In this review, we aim to evaluate recent advances in the use of electrochemical and optical biosensors for NDD detection.

1.1. Alzheimer's disease

AD is a disease of the central nervous system characterized by progressive loss of memory and other cognitive functions. Currently, there are 46 million people living with AD worldwide, and this number is expected to triple by 2050 [3]. The pathological hallmarks of AD include extracellular deposits of dense-core amyloid plaques that stain positively for thioflavin-S and Congo-Red, neurofibrillary tangles (NFT), cerebral amyloid angiopathy (CAA), neuronal and synaptic loss in the cortex [5]. The extracellular amyloid plaques result from the abnormal accumulation and deposition of Aβ peptides, Aβ40 (40 amino acids) and Aβ42 (42 amino acids), which are otherwise two normal protein by-products derived from the sequential cleavage of the *Amyloid* Precursor Protein (APP) by the enzymes β- and γ-secretases. Aβ is an amphipathic peptide of variable length ranging from 38 to 43 residues with the most common forms being 40 and 42 residues long. Although amyloid plaques composed of aggregated Aβ are the pathological marker for AD, abundant evidence points to the oligomeric intermediate within the Aβ aggregation pathway as the toxic species [6–8]. Studies have also shown that inheritance of E693Δ mutation *(Osaka mutation)* in Aβ**,** which inhibits the formation of insoluble amyloid fibrils and promotes the formation of soluble Aβ oligomers, results in a condition that closely resembles AD [9]. This has led multiple groups to focus on the toxic Aβ oligomers as a probable cause of AD.

Initially, it was postulated that amyloid plaques causally contributed to the onset and progression of AD. However, other lines of evidence have called this over-simplistic notion of AD pathology into question. For example, an influential 2003 paper by Giannakopoulos et al. [10], reported that NFT and neuron numbers, but not amyloid plaque loads, reliably predicted cognitive decline in AD patients. For instance, the role of Tau protein in NFT of AD brain has been intensely investigated [11–14]. Tau helps to maintain cell structure and function in healthy neurons by association with microtubules inside neuronal cells. In AD patients, however, tau is phosphorylated to a degree that causes dissociation from the cellular architecture and aggregation into insoluble NFT and paired helical filaments (PHF), which disrupt cell function and cause cell death [11,15]. Aggregation of tau involves formation of various soluble oligomers, which mature and aggregate further to form insoluble PHF and NFT. It is thought that these soluble tau oligomers are neurotoxic [12,16] while the insoluble mature PHF and NFT acts as nucleation points to remove soluble oligomers and thus serving a protective role [13]. Soluble tau oligomers are important drug targets but overall, targeting tau pathology remains a significant challenge [14].

There is also evidence for the involvement of apolipoprotein (apo) as a major risk factor in AD. ApoE is a polymorphic lipid-binding protein with three common allele variants: ApoE2, ApoE3 and ApoE4. ApoE4 gene is the strongest and the only confirmed genetic risk factor for the development of late onset AD, which enhances the risk level by three times in heterozygous individuals and by twelve times in homozygous individuals [17]. ApoE3 is the most common isoform with an incidence of 78%, E4 has an incidence of 15%, and E2 of 7% [18,19]. A high-risk E4 isoform of Apolipoprotein (ApoE4) appears to be associated with the amyloidogenic pathway [20,21]. The difference between these isoforms is a change at the 112 and 158 positions in the N-terminal domain. ApoE3 has Cys/Arg, ApoE4 Arg/Arg, and ApoE2 Cys/Cys. Structurally, this change in the primary sequence is known to decrease the stability of N-terminal helical bundle and promote an ionic interaction between the N- and C-terminal domains, which has been associated with the preference of ApoE4 to bind very low density lipoproteins (VLDL) over high density lipoproteins (HDL) [22]. Several in vitro studies have shown that the lipid-free ApoE4 bound Aβ with a higher affinity than ApoE3 and promoted fibril formation [23]. On the other hand, the association of ApoE3 with smaller oligomeric Aβ species may facilitate its exchange on lipoprotein particles at the blood-brain-barrier [22]. It is hypothesized that ApoE2 and ApoE3 are not high-risk isoforms due to the formation of disulfide bridge between Cys residues during dimerization. Because the presence of Arg at position 112 in ApoE4 (instead of Cys as in ApoE2 and ApoE3) drives an electrostatic docking of the C-terminal domain with the N-terminal helical bundle, this reduced C-terminal availability may explain the reduced binding affinity of ApoE4 for toxic oligomeric forms of Aβ. While ApoE4 is emerging as a promising biomarker, its reliability as an AD biomarker, especially, in early AD diagnosis and monitoring, has been called into question. For a more thorough analysis of AD biomarkers, please refer to review by Hampel et al. [24] Increasingly, it appears that AD is a multifactorial disease caused by the co-pathogenic interaction of multiple factors, including APP/Aβ**,** apoE4, tau, aging and various co-morbidities [25].

1.2. Parkinson's disease

PD is a chronic progressive neurodegenerative disease characterized by the loss of dopaminergic neurons in the brain region known as substantia nigra (SN), leading to clinical symptoms such as bradykinesia, rigidity, tremor and postural instability [26]. The pathological features of PD include 50–70% neuronal loss in the SN region, neuronal inclusions composed of α-synuclein protein located in the neuronal cell body - 'Lewy bodies' and neurites – 'Lewy neurites' [27]. There is growing evidence to show that dysfunctional regulation and mis-folding of α-synuclein in Lewy bodies is involved in the pathogenesis of PD [28–32] Oligomers of α-synuclein have also been proposed to be the toxic species responsible for neuronal death in the early stages of PD [30]. There is also compelling evidence for α-synuclein oligomer mediated neuronal toxicity caused by morphological changes to α-synuclein in the presence of metal ions [31,32]. Although PD is predominantly considered to be an idiopathic (sporadic) disease, there is strong evidence for the involvement of a number of risk factors. Leucine Rich Repeat Kinase 2 gene (*LRRK2*/*PARK8*) mutations are the most common cause of the 'familial' form of the disease, occurring with a frequency of 5–7% in patients with a family history of PD [33]. Other potential risk factors for PD, such as single gene mutations in *Parkin*, *DJ-1*, *SNCA* (α-synuclein gene) and *PARK4,* mitochondrial complex I abnormalities, dopamine (DA), microtubule-associated protein Tau gene (*MAPT*) have all been implicated in the pathogenesis of PD [34].

1.3. Prion diseases

Neurodegenerative diseases such as CJD, BSE, Kuru, Gerstmann-Straussler-Scheinker syndrome (GSS) and Fatal Familial Insomnia (FFI) belong to a class of prion diseases known as Transmissible Spongiform Encephalopathies (TSEs). TSEs are progressive, transmissible and ultimately fatal NDDs caused by the mis-folding and aggregation of a host-encoded cellular protein known as Prion Protein (PrP).