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Clinically oriented Alzheimer's biosensors: expanding the horizons towards point-of-care diagnostics and beyond

Bayu Tri Murti,[ab] Athika Darumas Putri,[bc] Yi-June Huang,[de] Shih-Min Wei,[de] Chih-Wei Peng[ef] and Po-Kang Yang *[deg]

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The development of minimally invasive and easy-to-use sensor devices is of current interest for ultrasensitive detection and signal recognition of Alzheimer's disease (AD) biomarkers. Over the years, tremendous effort has been made on diagnostic platforms specifically targeting neurological markers for AD in order to replace the conventional, laborious, and invasive sampling-based approaches. However, the sophistication of analytical outcomes, marker inaccessibility, and material validity strongly limit the current strategies towards effectively predicting AD. Recently, with the promising progress in biosensor technology, the realization of a clinically applicable sensing platform has become a potential option to enable early diagnosis of AD and other neurodegenerative diseases. In this review, various types of biosensors, which include electrochemical, fluorescent, plasmonic, photoelectrochemical, and field-effect transistor (FET)-based sensor configurations, with better clinical applicability and analytical performance towards AD are highlighted. Moreover, the feasibility of these sensors to achieve point-of-care (POC) diagnosis is also discussed. Furthermore, by grafting nanoscale materials into biosensor architecture, the remarkable enhancement in durability, functionality, and analytical outcome of sensor

[a]*Graduate Institute of Biomedical Materials and Tissue Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan*

[b]*Semarang College of Pharmaceutical Sciences (STIFAR), Semarang City, Indonesia*

[c]*Department of Pharmaceutical Sciences, School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan*

[d]*Graduate Institute of Nanomedicine and Medical Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan. E-mail: yangpk@tmu.edu.tw*

[e]*International Ph.D. Program in Biomedical Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan*

[f]*School of Biomedical Engineering, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan*

[g]*Department of Biomedical Sciences and Engineering, National Central University, Chung-li, Taiwan*

Bayu Tri Murti received his bachelor's in pharmacy from Faculty of Pharmacy, University of Jember, Indonesia. He then pursued his master's degree in Durban University of Technology, South Africa focusing on Alzheimer's biosensor and computational modelling. He is currently a PhD student in the Graduate Institute of Biomedical Materials and Tissue Engineering (GIBMTE), College of Biomedical Engineering, Taipei Medical University, Taiwan under the supervision of Prof. Po-Kang Yang and Prof. Chih-Wei Peng since 2019. His current research concentrates on the development of low dimensional nanomaterials-based biosensors for the detection of neurological biomarkers.

Athika Darumas Putri received her bachelor's in pharmacy from Faculty of Pharmacy, University of Jember, Indonesia. Afterwards, she completed her master's degree from Durban University of Technology, South Africa focusing on cancer aptasensor and computational modelling. She is currently a PhD student in the School of Pharmacy, Taipei Medical University, Taiwan. She specializes in the bio-nano interactions for the effective drug delivery systems and in silico studies.

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devices is presented. Finally, future perspectives on further translational and commercialization pathways of clinically driven biosensor devices for AD are discussed and summarized.

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1. Introduction

The prevalence of Alzheimer's disease (AD), 60–70% of dementia's total occurrence, has been increasing extensively worldwide. In 2016, 43.8 million people suffered from dementia, and these statistical figures are expected to even reach 100 million by 2030–2050.[1,2] AD corresponds to an extremely harmful and irreversible neurodegenerative disorder which can gradually cause serious brain problems.[3] Currently, the common approach to characterize AD is by various pathological markers, which include amyloid-β (Aβ) plaques, tau proteins, vascular damage, loss of synapses, damaged neuronal cells, formation of dystrophic neurites, noticeable gliosis, *etc.*[4–6]

To overcome AD, there are two major strategies being actively applied. The first strategy is to develop new biological and chemical entities for therapeutic usages. For instance, a great deal of effort has been reported to develop chemotherapeutic drugs and their delivery vehicles to effectively eliminate or target the disease biomarkers. However, serious limitations have been found from AD pathophysiology, lacking consensus on crystal-clear comprehension, and heterogeneity of responsible hallmarks.[7,8] More importantly, this strategy also restricts the development of highly selective medicines to clinically eradicate the biomarkers. In contrast, the second strategy is to establish biomarkers for early-stage detection and progression monitoring *via* engineered nanomedical approaches. Biosensor is a typical example, leading to the early prevention of the disease and avoid a more severe stage. This strategy has been widely accepted as an appropriate toolbox to overcome AD, which is coherent with the rapid observation on drug discovery and development.

Recently, biosensor technology has been largely explored in the various fields of neurodegenerative diseases.[9,10] Biosensors, as first terminology coined by Clark and Lyons in 1962,[11] is described by International Union of Pure and Applied Chemistry (IUPAC) as "a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues,

Dr Yi-June Huang received his BS and MS degrees in Department of Chemical Engineering at Chung Yuan Christian University, Taiwan, in 2012 and 2014, respectively. He received his PhD in Chemical Engineering at National Taiwan University, Taiwan in 2019. His research interests mainly surround applications in electrocatalyst materials for electrochemical systems, including dye-sensitized solar cells, water splitting, and energy storage devices.

Chih-Wei Peng received the B.S. degree in physical therapy from Chang Gung University, Taoyuan, Taiwan, in 2000, and the M.S. and PhD degrees in biomedical engineering from National Cheng Kung University, Tainan, Taiwan, in 2002 and 2007, respectively. He is currently a full Professor and Chairman at the School of Biomedical Engineering in Taipei Medical University. His research interests include neural engineering, biomaterial, biosignal process, biosensor, rehabilitation engineering, neural prostheses, urological electrophysiology, and functional electrical stimulation.

Shih-Min Wei received her master's degree from Graduate Institute of Biochemical and Biomedical Engineering, Chang Gung University. She is currently a research assistant in Graduate Institute of Nanomedicine and Medical Engineering, College of Biomedical Engineering, Taipei Medical University. Her research interests include biomaterials and tissue engineering.

Dr Po-Kang Yang received his PhD degree from Graduate Institute of Photonics and Optoelectronics, National Taiwan University. He is currently an assistant professor in Graduate Institute of Nanomedicine and Medical Engineering, College of Biomedical Engineering, Taipei Medical University. His research interests include the development of nanomaterials, energy harvesting, flexible electronics, self-powered nanosystems, and biomedical sensing platform.

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Table 1 State-of-the-art biosensors for the detection of Alzheimer's biomarkers and their transducing techniques. Accessed 16.02.2020

Alzheimer's biomarker	Transducing technique-based biosensors type																	
	Electrochemical	*Fluorescent*	*Immunosensing*	*Interferometric reflectance spectroscopy*	*Microelectrode*	*Organic electrochemical transistor*	*Ratiometric*	*Photoelectrochemical*	*Photonic*	*Plasmonic*	*qPCR*	*Surface-enhanced Raman spectroscopy*	*Microsized slit-embedded cantilever*	*Colorimetric*	*Filtration effect*	*Electrical*	*Electrochemiluminescent*	*Field-effect transistor*
Aβ	●	●	●	●	●	●		●	●	●			●	●	●	●	●	●
Tau	●	●	●					●		●								
α-synuclein	●																	
α-1 antitrypsin	●																	
Acetylcholine	●																	
Apolipoprotein E4 gene	●	●								●								
C-reactive protein	●																	
Dopamine	●	●																
Glycated albumin	●																	
Brain metal ions/metal ions	●						●											●
Melatonin	●																	
Micro-RNA	●										●			●				
Target DNA	●																	
Clusterin	●																	
Superoxide anion	●																	
Neurotoxin		●																
Glutamate					●													
Messenger RNAs									●									
O-linked-N-acetylglucosamine										●								
Vitamin B12										●								
Crocein orange G												●						
Whole biomarkers												●						
Choline														●				
Fetuin B and clusterin														●				
Glycogen synthase kinase-3 β																	●	

organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals".[12–14] Generally, biosensors consist of three parts. The first part is the biorecognition element, including antibody, peptide, enzyme, microbe, cell receptor, and DNA/RNA aptamer. Second, the transducer will convert the biological event to a measurable signal. Third, a signal processor will translate the signal into user-friendly display such as computerized graphics, diagrams, spectra, *etc.* The analytical performance of biosensors depends on sensitivity, specificity, selectivity, and accuracy. To reach an optimum level of sensor detection, various types of biosensors have been reported, such as electrochemical-, field-effect transistor-, plasmonic-, immuno-, fluorescent-, optical sensors, *etc.*[15–19] (Table 1). Nevertheless, some critical issues still remain unsolved from the recent findings. Particularly, how to enable device to effectively detect biomarkers at an early stage of the AD, sample invasiveness, and device miniaturization have become an urgent matter.

Today, biosensors have been addressed as the point-of-care (POC) diagnostics strategy for AD. It works as a screening platform instead of a conclusive diagnostic method. Tremendous studies have been conducted in this field.[10,20–23] Most of the

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constructed devices were applied to either blood or human cerebrospinal fluid (CSF) samples, while several reports employed minimally invasive samples, such as nasal secretions, saliva, *etc.*[24–26] The mainstream development is now directed towards POC device in which testable at the time and place of patient care, capable as a self-testing, as well as complying the ASSURED guideline: Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Delivered.[27,28]

In this review, we attempt to summarize forward-looking developments of promising detection strategies towards AD, especially for clinically driven biosensors design. The most recent development of biomarkers for AD, biosensor design and state-of-the-art biosensing strategies (*i.e.*, *in vitro* and *in vivo*) are presented. Moreover, the importance of *in silico* study to support the experimental findings is also briefly discussed (Fig. 1(A)). Finally, a perspective on the regulation of the biosensor development and commercialization pathways is also provided as future remarks. This aims to signify the emerging role of biosensor in current diagnosis for AD and to address their advancements towards future clinical applications.

2. State-of-the-art on clinically oriented biosensor for AD

Currently, the decisive cause of AD remains unknown. Many hypotheses have been associated with the Alzheimer's etiology including amyloid cascade hypothesis, tau propagation hypothesis, neurotransmitter hypothesis, calcium homeostasis hypothesis, mitochondrial cascade, neurovascular hypothesis, metal ion hypothesis, inflammatory hypothesis, exercise hypothesis, virus hypothesis, diabetes hypothesis, lymphatic system hypothesis, *etc.*[3] Among them, regardless of its recent failure in clinical trials, amyloid hypothesis has been regarded as the mainstream concept underlying AD research in the past two decades.[29] The solid consensus in this field will be beneficial in escalating the successful therapeutic interventions as well as in AD diagnosis.

In the scope of Alzheimer's diagnosis, there were notable reports on biosensor encompassing early detection and disease monitoring. Several biomarkers have recently been utilized such as amyloid, tau, apolipoprotein E4 (ApoE4) gene, α-synuclein, α-1 antitrypsin, acetylcholine, *etc.* The overview of these biomarkers is illustrated in Fig. 2. Motivated by the clinical need for biosensor-based diagnosis, the surveyed biosensors are divided into two main categories, *i.e.*, *in vitro* and *in vivo* biosensing systems. As for the *in vitro*, the biosensor device could be applied to test the samples in which the biochemical reactions occur outside of living body. The samples include human based-biofluids or artificial samples, cell culture, tissue excluding the living organism, and physiologically relevant pre-clinical analytes.[30,31] As for the *in vivo* methodologies, the biosensor device is simply operated inside the body or may be implanted into a living system.[32] Further, an insight into the biosensor architecture potentially used as POC diagnosis of AD is also briefly presented.

2.1. Biomarkers as crucial target for biosensor system

Biomarkers basically belong to proteins, enzymes, biological metals, genes, small biomolecules, and metabolites which have been largely employed as the bioanalyte in AD biosensor development (Fig. 2).[33] Aβ, tau protein, and phosphorylated tau are shown to be predominant markers for the AD.[33,34] Additionally, most of them are known to be accumulated in CSF and neuroimaging samples.[35] To date, scientists and clinicians have been working on developing advance tools of AD diagnosis by targeting the various type of biomarkers.[36,37] For example, the progress on biosensors based on specific Aβ detection,[10] and aptamer-based biosensors for AD have been reported.[38] However, it should be noted that a clinically oriented biomarker with appropriate biosensor design for AD has not yet been properly addressed. In the following, we will stress our focus to

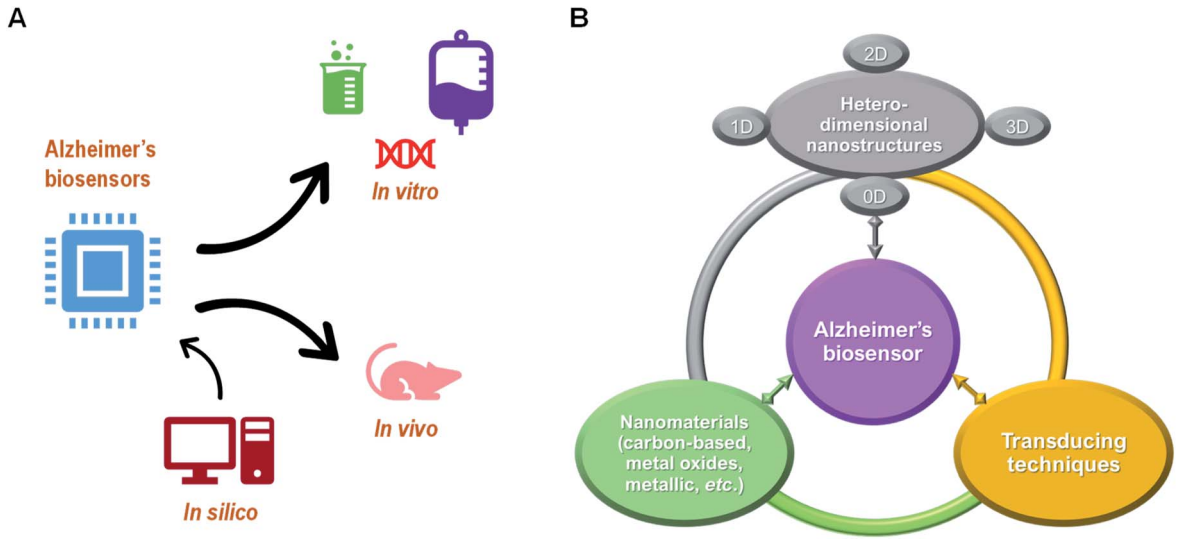

Fig. 1 (A) Clinical application of biosensor for AD and (B) their core elements towards modern diagnostic approaches.

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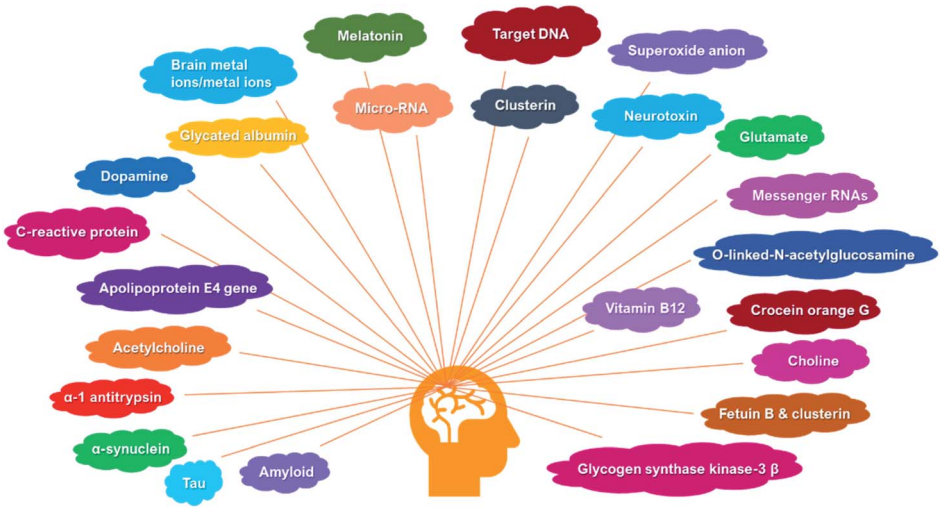

Fig. 2 Type of current biomarkers developed in biosensor platform for AD.

provide recent developments on biosensors for AD based on biomarker sources employed in pre-clinical and clinical settings.

(a) Aβ peptide. Traditionally, AD is marked by the coexistence of Aβ plaque depositions as it has been widely accepted as the most hypothetical marker during initiation and progression stage.[39,40] Aβ is a peptide comprising of approximately 40 amino acids on the basis of sequential cleavages of Aβ precursor protein (APP).[29] The origin of neurotoxicity in Aβ (40) peptides relies on the Aβ (residues 16–21) which is the central hydrophobic segment for the amyloid fibrillization known as the "amyloidogenic domain".[41–43] CSF contains different types of Aβ plaques, where the major compounds correspond to Aβ (40) and Aβ (42) isoforms. Other forms of peptides are also generated from APP in minor percentages.[44,45] Interestingly, accumulated amyloid can also be found in several minimally invasive biofluids including blood,[46] nasal secretion,[24,26] salivary gland biopsy,[47] and retina.[48] These recent studies have turned over the usage of conventional CSF-based biosensors due to less invasive sampling procedure, well-fitted with basic principle of POC diagnosis. However, it is noteworthy to mention that their amyloid loads may extensively differ from that of CSF as the gold standard of today's AD clinical diagnosis. For instance, Aβ concentrations are 10-fold higher in CSF than in blood plasma.[49] This challenge could be overcome through a proper design of biosensing devices such as truthful biorecognition elements, transducing techniques, and the use of microfluidic channel, as well as nanomaterials in supporting the sensor architecture (Fig. 1(B)).

In clinical setting, many diagnostic methods have been applied to detect Aβ. Traditionally, neuroimaging (*e.g.*, positron emission tomography, magnetic resonance imaging, and near infrared fluorescence) is the foremost clinical assessment tool for Aβ deposition *in vivo*. Its imaging quality depends on several factors such as the instrument resolution, the ability of contrast agent to surpass blood–brain barrier (BBB), its specificity towards Aβ diffuse, core, and intermediate plaques species.[50–52] In general, the use of neurological imaging has been limited within community owing to the high analytical cost and operational, and a poor understanding of amyloid burden relationship with cognitive dysfunction.[51,53–56] Instead, immunoassay method (*i.e.*, enzyme-linked immunosorbent assay (ELISA)) has been a plausible choice for the clinical diagnosis of Aβ. ELISA is the gold standard immunoassay for CSF Aβ (42) detection and is the most frequently used diagnostic method in AD.[36,52,57,58] Tremendous studies have reported the ELISA application in detecting Aβ levels in CSF, human plasma, and serum samples.[59–64] Numerous lab-based ELISA kits have now been available in the market with the detection range of 10 to 1000 pg mL^{-1} and proficient of discriminating AD patients with healthy control with good sensitivity (80%) and moderate specificity (83.3%).[52,65] In spite of time-consuming and laborious issues of traditional ELISA, the alternative ELISA kits have been widely developed for amyloid detection, which include paper-based ELISA[63] and digital ELISA.[66,67] Both of them are cost-effective, easy to operate, and portable and thus suitable for POC diagnostic approach.[52,65]

In addition to amyloid-based diagnosis, biomarker for AD is not only relied on Aβ molecules (*i.e.*, monomer), but also on their elongated forms[68] so-called Aβ oligomers (AβO) and fibrils. AβO has been regarded as the most neurotoxic entities and meticulously associated with the AD severity than that of insoluble Aβ aggregates,[69] specifically in the early phase of disease initiation. Their concentrations were observed to be up to 70-fold higher in AD as compared to non-demented controls.[70,71] The relationship between the Aβ assemblies and their toxicity was previously elucidated based upon the peptides binding with the fluorescent probes.[72–74] Due to the higher toxicity of AβO over amyloid fibrils, the detection of oligomeric amyloid is advantageous to precisely represent the progression of AD, particularly for its early stage.[75,76] Recently, an electrochemical biosensor design was reported to detect AβO in an *in vitro* environment using thiolated cellular prion protein peptide as bioreceptor.[71]

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Antibody is favourable entity to detect amyloid biomarkers owing to its high affinity to the designated antigen.[38,77] Recently, scientist employed another recognition element such as aptamer to selectively detect AD biomarkers.[38,78] Aptamer has offered many advantages to biosensor design, such as higher affinity (as compared to classical antibody), less expensive, non-*in vivo* production, reproducible system, smaller molecular size, and availability for wide range of analytes.[38] In general, aptamer is defined as short sequence of nucleotides (DNA or RNA) that primarily designed to mimic antibody function. Aptamers are three-dimensional bioreceptors with molecular size significantly smaller than conventional protein antibody.[38] This bioentity is produced by systematic evolution of ligands by exponential enrichment (SELEX) resulting in prominent-affinity and selectivity entities towards analytes. Meanwhile, aptamer is also a fascinating choice in clinically oriented biosensors due to their specificity, biocompatibility, non-toxicity, as well as non-immunogenic features.[38] As for the current biosensors of AD, aptamers are tenaciously used as an alternative of antibody setting especially for bioreceptor in detection strategy. For instance, aptamer is useful to overcome the Debye–Hückel screening effect in field-effect transistor (FET)-based biosensor (*vide infra*), which commonly hinder the electrical signal from the target molecules under high concentrations of such ions.[79] FET, integrated with proper biorecognition elements such as aptamer or antibody, is a unique and useful sensing device to detect biomolecular targets[80–82] offering many advantages such as real-time, highly sensitive, specific, and label-free transduction of biochemical signals.[83,84] In principle, the sensing mechanism of an FET device involves structural and functional integration of biorecognition element in which the selective interaction of bioreceptors and analyte produces changes in biophysical and biochemical signal. The signal is then transduced and amplified *via* a field-effect towards the signal display.[83,85,86] Aptamer is highly beneficial in this typical device since the biosensing is carried out under the physiological fluids[87] such as human CSF and blood. However, aptamer also possesses several limitations while embedded in biosensors, which include lack of high-quality aptamers for clinically important targets and non-specific binding of aptamer-surrounding environment.[88] To overcome these limitations, an appropriate biosensor design with functional biomarker is needed, such as the compliment of nanomaterial, sandwich-type aptasensor, antibody complex, and polymer inclusion.[89] Additionally, a major concern has been given upon the failure of amyloid in latest clinical trials in which scientist started to reconsider that predominant role of amyloid in AD pathogenesis.[90,91]

(b) Tau. Tau has been defined as state-of-the-art biomarkers for AD, instead of amyloid peptides. Tau is a microtubule-binding axonal protein that is highly expressed in cortical neurons.[92] While amyloid and tau protein are continuously acknowledged as the predominant markers in AD, the associative mechanism between both remains varied.[93,94] Recent biomarker studies indicate that Aβ accumulation is followed by synaptic dysfunction and increased phosphorylation and secretion of tau.[92,95] Indeed, Aβ propagates the tau pathology into the cortex through direct neuronal connections[96–98] (Fig. 3). Human AD cases with plaques and tangles demonstrate a noticeably increased formation and propagation of bioactive, high molecular weight forms of pathological tau relative to primary age tauopathy cases with tangles.[93,99] Specifically, tau is one of the useful biomarkers that is responsible for the formation of neurofibrillary tangles.[100,101] Lisi *et al.* have developed a SPR-based biosensor platform to detect human tau in the *in vitro* environment (*i.e.*, artificial CSF).[102] The key advantage of targeting tau for sensor technology is its low detection limit within clinical concentration range (pM, picomolar). Herein, instead of using Au NPs for SPR resonance, one dimensional multi-walled-carbon nanotube (1D-MWCNT) materials conjugated with tau antibody were selected to attain a sandwich-based bioassay. The as-fabricated assay is able to enhance the SPR signal of two order ($\sim 10^2$) folds compared to conventional unconjugated sandwich.[102] The platform enables the as-designed sensor system to detect the tau analyte down to 125 pM of limit of detection (LOD) with the 125–1000 pM linear working range. Additionally, they extended similar study by further employing a non-SELEX-based aptamer to sense multi-tau biomarkers on a chip by using fluorescent anisotropy.[103] This remarkable platform can detect t-441, t-381, t-352, and t-383 isoforms with the LOD of 28 nM, 3.2 nM, 6.3 nM and 22 nM, respectively.

2.2. *In vitro* biosensing system

A versatile biosensing device, such as a commercial glucometer or alcohol meter, merely initiated with the assay at a non-natural sample and animal biofluids. In other words, the biochemical reaction, as a result of analyte and biorecognition element interaction, occurs outside of a living organism under the *in vitro* environment, such as a culture dish, a test tube, a microtiter plate, *etc.*[104] Nowadays, significant advances from nanotechnology and microfabrication process have shed light on the development of *in vitro* biosensors. Take microfluidic device as an example, the devices fundamentally combine

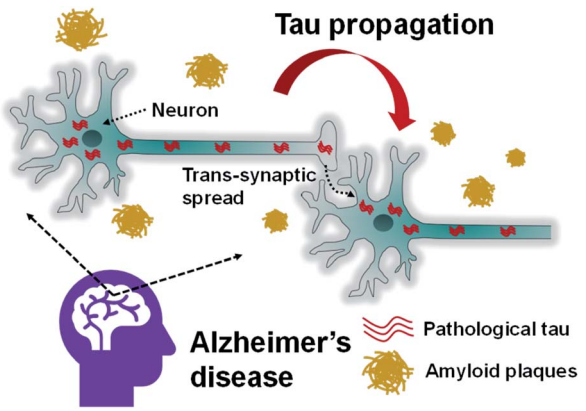

Fig. 3 Schematic depiction of cell-to-cell tau spreading in AD brain *via* neuronal trans-synaptic transmission. Adapted from ref. 98, open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

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miniaturized features of computing chip with living cells and tissues mimicking the human biology.[105] Additionally, organ-on-chips device have also been well-established simply to impersonate the more complex organ systems, since the excellent capability of the biosensors and microfluidic system.[106,107] These advances make the *in vitro* environment to be one of the plausible choices for biosensor development.

In vitro biosensor, as part of *in vitro* diagnosis (IVDs), refers to the assays or tests that are carried out on samples such as blood or tissue withdrawn from the human body. By regulation, the IVDs is defined by U.S. FDA as the specific subcategory of medical devices comprising "those reagents, instruments and systems intended for the diagnosis of disease or other conditions". Relevant diagnostics are then suitable with this category, which include a determination of the state of health, cure, mitigation, treatment, and prevention.[108] Moreover, IVDs may also be used in precision and personalized medicine to identify the patients benefitting from a particular therapies and treatment.[108,109] In 2016, the global diagnostics market was counted as US$40–45 billion with POC diagnostics supplying US$12–13 billion. The annual growth rate of IVDs is forecasted to be 5%.[110,111] The rapidly growing market of IVDs emphasize the prominence of this technology, not only for academia-based research but also in industry. Recently, the global pandemic of COVID-19 also fuels the research and development in pursuit of IVDs technology, especially for cardiovascular diseases, neurological diseases, liver dysfunction, pneumonia, *etc.*

For the sensing techniques driven to *in vitro* analysis, several efforts have been conducted for biosensor design targeting AD.[112] For example, a state-of-the-art soft material fabricated with DNA origami has been introduced as artificial peptide nano-network biosensor to tackle the pathological peptides aggregates in neurodegenerative disease, by imitating pathogenesis process.[31] Particularly, periphery platelet enables itself to secrete amyloid proteins and initiate their cross-linking to establish a surface peptide molecular-based system. This platform could sophistically discriminate the AD patients from healthy volunteers by detecting potential neurodegenerative activity of platelet with the <1 pg mL^{-1} detection limit and 3.3–3300 pg mL^{-1} dynamic range, which is superior to ELISA method. This biosensor is potentially used towards the label-free and early screening (IVDs) of AD biomarkers circulating in minimally invasive blood sample. Table 1 and Fig. 4(A) show the distribution status of state-of-the-art biosensors for AD, the cutting-edge usage of biosensors in anchoring from forefront analyte recognition towards signal amplification and data acquisition.[113] To effectively diagnose AD, various techniques have also been developed towards specific biomarker and sensing strategies, such as fluorescent biosensors, immunosensors, electrochemical biosensors, field-effect transistor-type biosensors *etc.* (Table 1 and Fig. 4(A)).[114] Among them, it is noted that electrochemical biosensor has attracted prodigious attentions (*i.e.*, 43.18% in Fig. 4(A)). More interestingly, *in vitro* platform occupies the major portion of biosensor development for AD, counting over 95% (Fig. 4(B)).[114] The reason could be due to the intricate procedure of *in vivo* biosensor which commonly need to be implanted into the living animal model. Meanwhile, *in vivo* biosensors also need to concern with the biocompatibility, systemic toxicity, biodegradability of the materials used, that make *in vivo* biosensors more complex to achieve in comparison with *in vitro* ones.[115–117]

(a) Electrochemical biosensors. Electrochemical biosensor has been widely applied to biomarker detection due to its capability of achieving extremely low detection limit down to attomolar regimes.[118–127] An electrochemical biosensor is a self-contained integrated device, which provides quantitative or semi-quantitative analytical data on the basis of interaction between bioreceptor retained in direct spatial contact with an electrochemical transduction element, with the target analyte(s).[13] This platform transduces the biochemical events into electrical signals that is further translated into a readable display.[128–130] Since the first electrochemical sensor introduced by Clark and Lyons to measure the blood glucose,[11] the diverse forms of biosensor have been established in various applications.[130]

Several types of working modes have been well-established in electrochemical sensors *via* amperometry,[131] voltammetry,[132] impedimetric,[133] conductometry,[13] and interdigitated microelectrode techniques.[24] The advantages of this sensing platform mainly rely on two aspects *i.e.*, capability of device miniaturization and cost-effective instrumentation.[134] More importantly, some commercialized biosensors have become popular owing to these merits (*i.e.*, POC glucose sensors and alcohol monitoring device).[134,135] Meanwhile, it is also noted that electrochemical biosensors have been utilized for routine analysis paving the way for POC diagnostics of AD.[10,22,23]

As for the diagnosis of AD, an electrochemical biosensor platform was recently reported to detect AβO in an *in vitro* environment using thiolated cellular prion protein peptide as bioreceptor.[71] Moreover, Sun *et al.* also employed composite hetero-structured nanomaterials and three-dimensional (3D) hydrogel to achieve 0.1 pM detection limit in 0.1–10 nM linear working range, complying the clinical diagnostic concentration of amyloids in human plasma and CSF (5.5–195 pM). This 3D-hydrogel biosensor composed of graphene oxide (GO)/Au nanoparticles (Au NPs) provided significantly larger surface area as compared to solid electrode allowing rapid penetration of target biomolecules towards bioreceptor binding moiety. The purpose of utilizing GO in hydrogel is to contribute towards the tunable conductivity and bionic structure of the electrode.

Additionally, a differential pulse voltammetry (DPV)-based electrochemical biosensor was employed by Negahdary and Heli to detect Aβ (42) in an artificial CSF and spiked serum samples.[136] This strategy embedded Aβ (42)-binding peptides on the microporous Au nanostructure, and successfully achieved attomolar (aM) level of detection limit *i.e.*, 0.2 pg mL^{-1} (44.3 aM), with a linear working range of 3–7000 pg mL^{-1}. Authors further extended the similar work with the use of different biorecognition element *i.e.*, 107-mer thiol-modified RNA aptamer.[129] However, this study represented the slightly higher LOD (*i.e.*, 0.4 pg mL^{-1} or 88.6 aM) than that of antibody setting. The results indicate the better compatibility of antibody with the electrochemical-based strategy, in particular for amyloid detection, as compared to aptamer. Indeed, owing to the high

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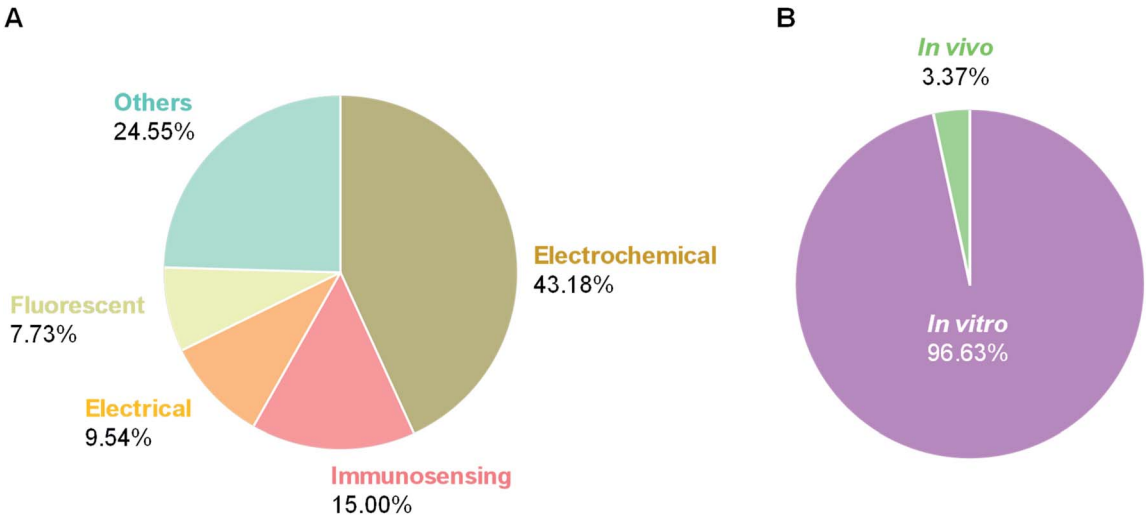

Fig. 4 (A) Total publication status in Alzheimer's biosensor during 2017–2020 (information obtained from Scopus database. Accessed on 09.12.2020). (B) Distribution of *in vitro* and *in vivo* biosensors for AD (information obtained from PubMed database. Accessed on 16.02.2020).

surface area during immobilization process, the addition of Au nanomaterials in those studies are another key medium towards the ultrasensitive biosensors device.[129]

Another electrochemical biosensing platform was designed to detect Aβ in its oligomer by using brain samples of normal mice and with AD.[137] The label- and antibody-free biosensors were fabricated for the AβO detection employing cellular prion protein as a receptor for AβO yielding detection limit at picomolar level (10^{-4} pM). In contrast to the classical ELISA method, this platform used PrP^C receptor to capture the amyloid target as opposed of antibody suffered from time-consuming procedure and lengthy incubation. Additionally, an electrochemical strategy was developed to measure AβO *in vitro* acquiring a few μM LOD by dual transducing techniques, where CV and UV-Vis spectrophotometry in human blood serum and artificial CSF.[138] This approach utilized the competitive nature of Zn ions and AβO releasing ferrocene from its Zn zeolitic imidazole framework which then subsequently detected by the transducers. This study protocol provided a decent feasibility of using artificial sample comprising biomarker of AD.

Actually, instead of amyloid-based marker, melatonin could also be detected by electrochemical method with the prime marker source from rat's liver extracts (spiked with melatonin).[132] Melatonin abnormal circulation have been correlated to several diseases such as AD, type II diabetes mellitus, and several types of cancers.[132,139] This melatonin-targeted immunosensor was reported as easy-to-use device for rapid quantification of melatonin reaching micromolar LOD, which can be further potentially used as a POC device in the future.[132] Other sensing techniques were also successfully employed in developing electrochemical biosensor of AD with animal-sample tests, which include microelectrode array (MEA),[140] ratiometric photoacoustic nanoprobe,[141] and electrochemiluminescence.[142]

As widely understood, tau protein is also an excellent candidate for human sample-based biosensor for AD. An electrochemical biosensor was recently developed to detect tau-381 based on aptamer–antibody sandwich assay in human serum from patients with AD.[143] DPV technique was used herein as quantification method resulting in LOD of 0.42 pM with 0.5 pM to 100 pM dynamic range. This biosensor combines the advantages of prominent affinity of aptamer with the signal amplification of the Au NPs yielding ultrasensitive detection means for tau protein in real human serum. Meanwhile, an electrochemical sensor based on a single bioreceptor, aptamer, immobilized in carboxyl graphene nanomaterials, thionin, and Au NPs modified glassy-carbon electrode was subsequently introduced.[144] However, the LOD in this work was found to be slightly higher (0.70 pM) than their previous report (0.42 pM). Nevertheless, both reports emphasize the capability of electrochemical based sensors as an early screening of AD, particularly for clinically relevant sample (*i.e.*, real blood from AD patients). Further, it is noted that other biomarkers were also useful in human sample-based biosensor, such as ApoE4 gene from four genomic DNA samples extracted from human blood.[145]

Despite technologically convenience and advantage offered by electrochemical method, the biomarker detection using this technique still exists several limitations and challenges that need to be overcome. For instance, the as-designed device usually lacks selectivity since the reference electrode limits the charge carriers,[146,147] sensor instability over prolonged storage time, limited design for multianalytes detection, and lack of regenerative sensor devices.[148] The further development should aim at addressing these issues and simultaneously maintain the forefront characteristics of electrochemical biosensors such as high sensitivity, device miniaturization capability, and relatively cost-effective in mass production.

(b) Fluorescent-based biosensors. Fluorescent-based techniques are highly sensitive, efficient, and specific for biomolecules detection.[149,150] Conventional optical fluorescence, however, exhibits several limitations as a POC diagnostic strategy due to the lack of portability, high cost, requirement of a specific proficiency from the expert, and unsuitability in

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ambulatory environment.[150] To addresses these issues, a great effort has been made to develop a compact, portable, multiplexed, and cost-effective fluorescent modalities[151–156] such as smartphone embedment,[157] fluorescent lateral flow assay[158] *etc.*

As for the fluorescent-based AD biosensors, series of biomarkers have been employed as an *in vitro* setting such as amyloid,[159–161] dopamine,[162–170] neurotoxin,[171] tau,[172] and ApoE4 DNA.[173] The major targeted analyte in this type of biosensor is AβO considered as the main culprit for AD initiation and progression. By quantifying AβO in CSF and plasma, it is found to be beneficial for determining the disease severity.[174,175] In 2017, Jiang *et al.* designed a fluorescent biosensor to detect AβO in artificial CSF by employing Fe_3O_4 NPs and $BaYF_5$:Yb, Er upconversion NPs (UCNPs) as vastly sensitive labels, incorporated with the AβO aptamer and its complementary oligonucleotide.[159] The sensing performance of such a biosensor was shown by the 0.2–15 nM of linear working range and 36 pM of LOD. The addition of uniquely UCNPs and Fe_3O_4 NPs with aptamer can contribute to the picomolar sensitivity of the platform, particularly under artificial physiological surface. More studies are required to confirm the merit of this biosensor design to detect AβO in the relevant clinical samples *i.e.*, CSF and blood plasma.

Similar target was also detected by a quench body technique (denoted as Q-body) embedded to a fluorescent sensor in an *in vitro* environment.[160] Q-body is a new kind of strategy to detect a broad range of biomolecules employing fluorescence quenching of the dye(s) attached to the antibody fragment.[176] Previously, Aβ-derived diffusible ligand (ADDL) was sensed by the double-labelled Fab type Q-bodies (the heavy and light chains) with a higher sensitivity than the Aβ peptides suggesting the promising usage of Fab type Q-bodies as a notorious bioimaging tool.[160] It should be noted that fluorescent-based technique also possesses several drawbacks, particularly in detecting the biomarkers of AD. On the one hand, label-based fluorescent biosensing measurement is often time consuming, cost-intensive, and possibly blockade the active binding sites in recognizing the targeted analytes. On the other hand, fluorescent-based technique would potentially affect the affinity-based interaction of bioreceptor and the biomarkers.[177] Nonetheless, this method has been widely used as "gold standard" in clinical setting particularly for monitoring of early stage of Aβ nucleation owing to its robust staining properties and method's conveniences.[178,179]

In terms of clinical perspectives, fluorescent technique has also been reported as proficient transducers to sense AD biomarkers in human samples.[180] Rajasekhar *et al.* reported a near-infrared (NIR) fluorescent-based biosensor constructed to detect amyloid aggregates in human brain tissue sample.[181] The authors used coumarin–quinoline (CQ) conjugate as the fluorescent probe exhibiting ~100-fold fluorescence power *in vitro* once bound to Aβ aggregates with augmented quantum yield. CQ showed non-toxic properties for neuronal cells and excellent permeability against BBB. CQ probe also demonstrated unequivocal selectivity towards the target protein as compared to other toxic protein aggregates such as tau, α-synuclein, and islet amyloid polypeptide in human brain tissue. This was currently regarded as a reliable method in distinguishing AD from tau pathology and mixed dementia. Uniquely, this study also reported *in silico* approach *i.e.*, density functional theory (DFT), molecular docking, and molecular dynamics, to elucidate the binding characteristics of ThT (control) and CQ within Aβ (42) fibril and further define the binding constant as compared to experimental data. Molecular structure of CQ and selective staining of Aβ plaques in human brain tissue are represented by Fig. 5(A) while the staining on the same plaque without NFTs of tau and the neuritic component (stained only with Tau phos Ser396/Ser404 (PHF1) antibody) are depicted by part (B) and (C), respectively. Nevertheless, the overlaid image indicated there was no colocalization of PHF1 and CQ compound (Fig. 4(D)). In this study, *in silico* study is beneficial to elucidate the lowest energy configuration of ligand (ThT or CQ) and protein (Aβ (42)) binding (Fig. 5(E)), to characterize the chemical bonding status, which is responsible for a binding motif (ligand–protein) and regarded as a validation of the experimental finding.[181–183]

Zhao *et al.* also reported a fluorescent-based aptasensor to detect amyloid markers by an employment of double stranded DNA (dsDNA)/GO as the fluorescent probe yielding LOD down to 0.1 nM with a linear detectable range from 0.1 nM to 40 nM.[184] This technique could discriminate the sample from AD patients and healthy persons indicating its acceptable selectivity. Moreover, it is noted that GO has effectively participated in reducing the non-specific adsorption due to its large surface area which provide the enormous covalent conjugation with Aβ (40) oligomers-targeting aptamer and therefore,

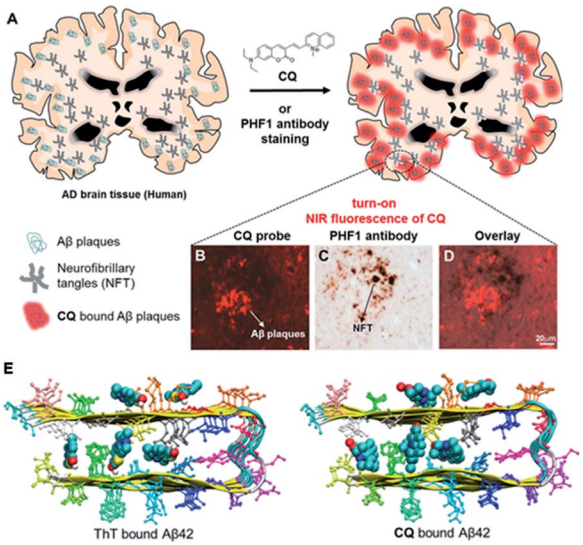

Fig. 5 (A) Chemical structure of CQ and selective staining of Aβ plaques in human brain tissue. (B) CQ stains the plaques in the Alzheimer's brain tissue while NFTs of tau were not detected. (C) The neuritic component in the same plaque is only stained with Tau phos Ser396/Ser404 (PHF1) antibody. (D) The overlaid image demonstrated no colocalization of PHF1 and CQ compound. (E) Binding sites for ThT and CQ within Aβ (42) fibril (12 mer assembly) observed by *in silico* studies. Reproduced from ref. 181 with permission. Copyright (2017) Elsevier.

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improved the sensitivity and specificity of the device.[184] The role of nanomaterials in recent biosensor architecture is crucial to provide a high-performance sensing device in which for now and in the future, these will likely become a promising platform in disease screening as well as POC diagnosis of AD. Additionally, some recent studies also provide detailed explanation and strategies of fluorescent-based based biosensor for AD under *in vitro* environment,[185–188] due to their sensitivity and generally non-invasive.

(c) Plasmonic-assisted biosensors. Plasmonic-assisted sensor is one of the profound transducing methods of AD marker detection. For the plasmonic-assisted sensors, several biomarkers have been recently detected *in vitro* in correlation with AD *viz.* Aβ, ApoE4 gene, vitamin B12, and tau proteins.[189] Capability of quantifying biomarker panel (more than one marker) is apparently one of the key futures of the successful diagnosis of AD. Since the recent data emphasize that AD is associated with multi-factorial markers, obtained from "omics" studies, particularly on its pathophysiological steps.[190,191] A shape-code biosensor, an Au NPs-shape dependent embedded in distinct Localized Surface Plasmon Resonance (LSPR) was fabricated to detect multi-analyte of AD's biomarkers in artificial blood sample.[21] The LSPR is defined as an optical phenomenon resulted from light when it contacts with conductive NPs with a smaller size than the incident light wavelength. The Au NPs and antibody were governed as recognition elements resulting in LOD of 34.9 fM, 26 fM, 23.6 fM for Aβ (40), Aβ (42), and tau protein, respectively, along with a broad linear working range from 1×10^1 to 1×10^8 fM. This designated biosensor was denoted as the first shape-code biosensor for the detection of AD biomarkers. Likewise, another recent study demonstrated the hybrid mode of both plasmonic and photonic sensors capabilities.[192] An obvious light–matter interaction is allowed by this multiple biomarkers-based biosensor. This dual-transducing strategy emphasizes the ability of plasmonic method in the detection of multiple analytes in diagnosing AD. Moreover, several biomarkers were also detected by plasmonic method, particularly by size-, composition-, and shape-dependent LSPR.[193] The tremendously intense and localized electromagnetic fields induced by LSPR create an exhibited change in extinction and scattering spectra shifts resulting in highly sensitive transducers of NPs in the local refractive index.

A biosensing platform based on LSPR on 2D-photonic crystal (2D-PC) and Au-coated 2D-PC was constructed to detect AD-linked DNA oligonucleotide associated with ApoE4 gene sequence *in vitro*.[194] ApoE gene is known as a gene which responsible for Alzheimer's progression.[195] The use of this modified LSPR in this study was able to enhance specificity of ApoE gene detection resulting in a promising proof-of-concept for the miniaturized and wearable biosensors in numerous diagnostic and defence applications.[194] An interesting part of LSPR is the capability to detect analyte down to single nanoparticle as well as probing tremendously small volumes down to very low LOD achievement and cost-effective device in sensing avenue. However, despite the tremendous merits of LSPR assay in biosensing fields, their practical and clinical applications are still limited.[196] This may gain further concerns, specifically when this transducing method is being translated towards clinical diagnosis.

(d) Photoelectrochemical biosensors. Photoelectrochemical (PEC) method is another alternative for the tau-based biosensing owing to its femtomolar detection limit capability.[127] In PEC analysis, excited charge carriers are generated by a photoelectrode *via* harnessing light energy in which the photogenerated charges would be transported to the counter electrode through the external circuit. The minority carriers subsequently initiate redox reactions with sacrificial scavengers at the semiconductor or electrolyte.[197] A PEC-biosensor was developed to detect tau proteins using bismuth vanadate ($BiVO_4$) as an artificial electron donor-free.[127] The sensor was fabricated by integrating molybdenum (Mo) dopant and iron oxyhydroxide (FeOOH) ad-layer into the $BiVO_4$ photoelectrode and using horseradish peroxidase (HRP)-triggered oxidation of 3,3′-diaminobenzidine (DAB) as signal amplifier (Fig. 6(A)). Regardless of the absence in additional electron supplies, the FeOOH/Mo:$BiVO_4$ associated with the Tau5 antibody was able to generate strong current signals at 0 V under the white light-illuminated diode. The LOD and limit of quantitation (LOQ) of this approach were found at 1.59 fM and 4.11 fM, respectively. Accordingly, the LOD was compared with other values from electrochemical methods reported in literature (Fig. 6(B)) and tend to be the lowest among the designated studies.

(e) Field-effect transistor biosensors. Among various techniques used to detect the neurodegenerative biomarkers, a special interest is given towards FET-based sensors owing to their ultrasensitivity, wide ranging analytes detection, label-free, selectivity, reusability, and real-time detection capability up to living cells monitoring.[198,199] Silicon nanowire (SiNW)-based FET is one of the most developed field-effect devices, pioneered by Charles M. Lieber's group in 2001,[200,201] particularly for biological detection. Several *in vitro* biosensing measurements were demonstrated for the detection of Aβ (40) and metal ions associated with AD biomarkers. SiNWs-FET device was reported to be a functional device for amyloid detection with the use of aptamer as recognition element.[202] The binding mode of Aβ aptamer–Aβ showed in the range of 0.1 pg mL^{-1} to 10 μg mL^{-1} with the ultra-low detection limit (*i.e.*, ∼20 fM) due to the single-trap phenomena revealed by the novel SiNWs-FET structures. SiNWs-FET was also able to detect and quantify extracellular Zn^{2+} associated with neurotransmission and Aβ fibrillation by using Zn^{2+}-sensitive fluorophore, FluoZin-3, in a real time *in vitro* environment (*i.e.*, cultured cortical neurons).[203] This strategy can serve as a highly sensitive device *via* examining how Zn^{2+} homeostasis modulates neuronal activities. Furthermore, it can be a useful strategy in neurodegenerative disease prevention.

In addition to the great attempts made by using 1D nanomaterials in diagnosis platform for AD, two dimensional (2D) nanomaterials serve as highly promising materials for FET biosensors owing to their fruitful structural and electronic properties,[199,204] such as large surface-to-volume ratio, high electrical conductivity, fast electron transfer kinetic reaction,

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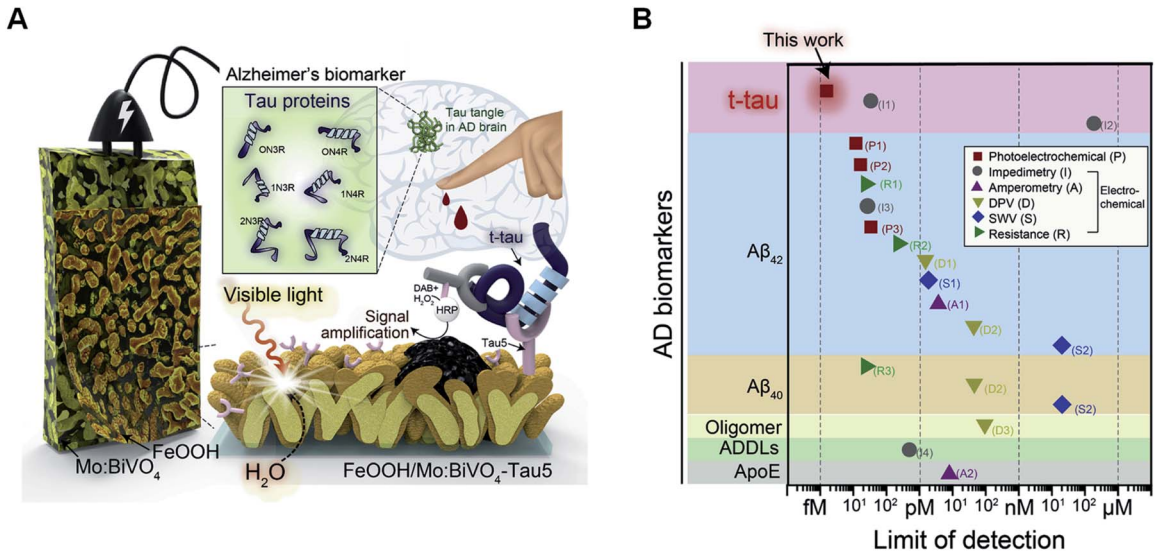

Fig. 6 (A) Representation of water oxidation-coupled, FeOOH/Mo:$BiVO_4$-based PEC sensing platform for the detection of femtomolar levels of tau. (B) LOD of Alzheimer's biomarker-targeting sensing platforms reported in literature. Reproduced from ref. 127 with permission. Copyright (2020) Elsevier.

and easy functionalization.[205] For instance, a graphene-based FET (G-FET) was fabricated to monitor the Aβ aggregation on the basis of ganglioside G_{M1}-enriched supported lipid bilayer (G_{M1}*-SLB/G-FET) (Fig. 7(A)).[206] The as-fabricated G-FET device is capable of detecting and monitoring the early nucleation phase of amyloid formation. It shows a larger potential as a promising biomimetic sensor to investigate membrane-related protein functions and interaction kinetics, as compared to ThT assay. Indeed, concurrent detections of the Aβ40 aggregation by both G_{M1}*-SLB/G-FET and ThT assays may benefit in future diagnosis of AD (Fig. 7(B)).[206] However, the ability of device to monitor the amyloid in the real sample (CSF or plasma) remains challenging with the presence of other interference proteins. Another vast obstacle of FET in clinical biosensing measurement relies on the high concentration of salts/buffers in clinical sample-induced Debye–Hückel screening effect.[79]

Debye–Hückel effect is the discussion about the correlation of Debye length and unambiguous selective detection of macromolecules. Debye length (λ_D) corresponds to the distance measured from FET-biosensor surface and electrolytic buffer solution (*e.g.*, phosphate-buffered saline) describing the screening of surface charges by ions in an electrolyte solution. Debye length is described as the eqn (1) below,

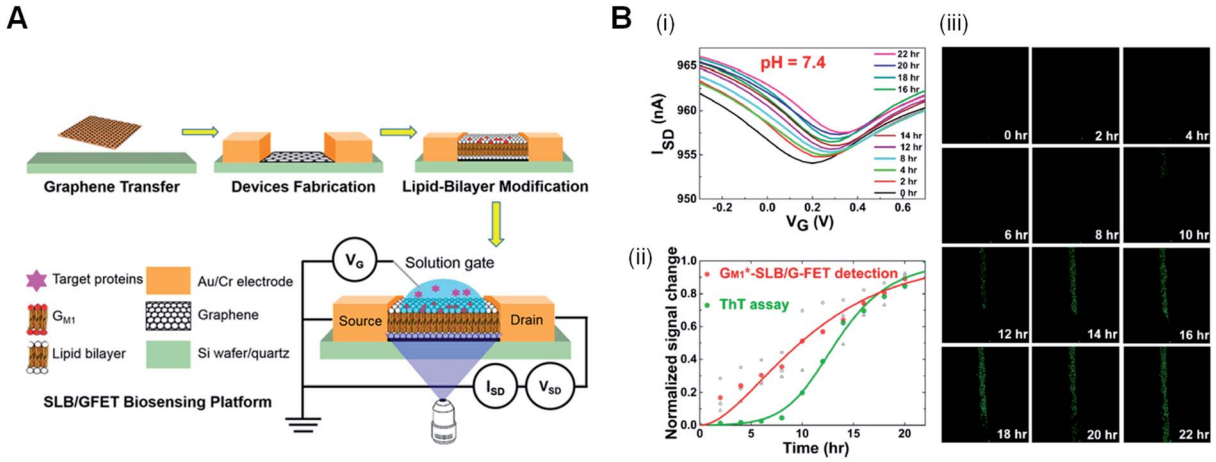

Fig. 7 (A) Schematic illustration of preparing a SLB/G-FET device with a solution-gate electrode. (B) Simultaneous detections of the Aβ (40) aggregation by both G_{M1}*-SLB/G-FET and ThT assay. (i) The gradual aggregation of the negatively charged Aβ (40) induced a positive doping to the device. (ii) ThT assay on the similar G_{M1}*-SLB/G-FET device where the fluorescence images were attained by collecting 450–550 nm emission from the ThT dye excited at 405 nm. (iii) Comparison of the observed signals by ThT assay (green dots) and G_{M1}*-SLB/G-FET (red dots) during the Aβ (40) aggregation. Reproduced from ref. 206 with permission. Copyright (2020) American Chemical Society.

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$$\lambda_D = \sqrt{\frac{\varepsilon_0 \varepsilon_r k_B T}{2 N_A e^2 I}} \quad (1)$$

where the ε_0 represents the vacuum permittivity, ε_r is the relative permittivity of the medium, k_B is the constant of Boltzmann (1.380649×10^{-23} J K^{-1}), T is the absolute temperature (298 K), N_A is the Avogadro's number ($6.02214076 \times 10^{23}$ mol^{-1}), e is the elementary charge, and I represents the ionic strength. Ionic strength (I) is further given as follow (eqn (2)),

$$I = \frac{1}{2} \sum_{i=1}^{n} C_i Z_i^2 \quad (2)$$

where C_i represents the molar concentration (mol L^{-1}) of the ion i, Z_i is the charge number or valence of ion and the sum is taken over all the ions in the buffer solution. Another equation which also can be used to calculate the λ_D is given by,

$$\lambda_D = \frac{1}{\sqrt{4\pi l_B \Sigma_i \rho_i Z_i^2}} \quad (3)$$

where l_B is the Bjerrum length (0.7 nm), Σ_i is the sum of all ions, and ρ_i and Z_i are the density and valence, respectively, of ion species i.[207] A more simple equation can be used as described below (eqn (4)),

$$\lambda_D = 0.32(I)^{-1/2} \quad (4)$$

From these equations, the most important parameter is ionic strength (I) which directly determine the λ_D. The selection of biorecognition element is also crucial for FET biosensor, particularly for the needs to be applied to relatively high-salt biological samples, such as blood, CSF, sweat, *etc.* This effect may lead to the clearance of electrical signal produced by target–receptor binding affinity.[198] Also, it is suggested that the dimensional size of recognition elements need to be below λ_D in order to omit the Debye–Hückel screening effect. Instead, another general approach is by diluting the sample into several orders of concentration to increase λ_D.

Towards next-stage of pre-clinical based-*in vitro* biosensors, the use of animal biofluid is the crucial steps of biosensor translational efforts, particularly for AD. As the frequent usage in electrochemical transducer, a recent immunosensing approach was developed to detect Aβ (40) fragment in brain tissue lysates prepared from AD-induced rats.[133] The devoted impedimetric micro-immunosensing assay used monoclonal Aβ (40) antibodies which were immobilized on a disc-shaped microelectrode surface connected to an impedimetric signal transducer resulting in 4.81 pg mL^{-1} (~1.11 pM) LOD with a dynamic range of 1–10^4 pg mL^{-1} (~0.23–24.02 pM). The constructed platform yielded a lower LOD as compared with the designated conventional ELISA method. On the other hand, the amyloid protein together with Cu^{2+} ions were capable of being detected in plasma and hippocampus of rats with normal and AD by a single ratiometric platform with biological processes association and direct involvement of Cu^{2+} ions in Aβ (42) aggregation.[208] This dual detection mode was achieved by detection of Cu^{2+} ions through neurokinin B and the Cu^{2+} releasing from a complex while the ions tend to bind the Aβ (42) protein in the solution. The detection limit was observed as low as 0.04 μM for Cu^{2+} and 0.5 ng mL^{-1} (~1.108 μM) for Aβ (42).

In vitro measurement could also be carried out by using various transducing methods, such as interferometric reflectance spectroscopy,[209] organic electrochemical transistor,[210] surface-enhanced Raman spectroscopy,[211,212] and quantitative polymerase chain reaction.[213] These transducers can provide clinically relevant-LOD concentration, simplicity, and reasonable cost of production to an extent by depending on the targets in analyte–bioreceptor interaction type and their native environment. However, further studies are still required to confirm the selectivity and specificity of the device towards real clinical samples.

2.3. *In vivo* biosensing system

In vivo biosensors have been treated as one of the future technologies of personalized healthcare. An implantable biosensor in human body can deliver essential healthcare information of the patients by continuous monitoring basis which are beneficial for reducing prolonged clinical procedures. This type of sensing platform is particularly important for those who need continuous healthcare monitoring. A small fluctuation will be easily captured and further provide the "health status" of one which further designate the following necessary treatment or therapy. For the life-threatening disease such as cancers, cardiovascular, and nondegenerative disease, the *in vivo* biosensor may serve as "baseline"-associated device for the prevention of further serious problems or complex outcomes. Another scenario is to continuously monitor chemotherapeutic medicine which can yield the guesswork out of dosing by displaying an individualized report on the pharmacokinetics. Despite many reports in *in vivo* biosensors towards this direction, only few reports addressed preclinical studies or further approved for human implantation.[116]

2.4. Current *in vivo* strategies of biosensors for AD

The use of electrochemistry to measure electroactive neurotransmitters such as dopamine, serotonin, norepinephrine, and their metabolites,[163–170] in whole animals is pioneered by Ralph Adams and his colleagues during the early 1970s[214] in which the dysregulation of these species can lead to AD.[215–217] Since then, the field has emerged as one of the important facets in microsensor[218] and real-time biological events monitoring, particularly for living tissue environment[167,219–221] and single-cell analysis.[164,222,223]

Indeed, conventional electrochemical techniques, *i.e.*, voltammetric, impedimetric, amperometry, and potentiometric, preserve a modest strategy towards Aβ detection *in vivo*.[224,225] Voltammetric method tends to be the most frequently used herein. The sensor merely measures current–potential relationship in which the potential represents as fingerprint-like electrochemical parameter for the determined species while the current is directly proportional to the species. The voltammetric family includes differential pulse voltammetry (DPV), cyclic voltammetry (CV), stripping voltammetry, AC voltammetry, linear sweep voltammetry (LSV), polarography, *etc.* Among

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in vitro and *in vivo* biosensing techniques, DPV seems to be favourable in measuring amyloid biomarkers, particularly for quantitative analysis. Whilst, CV has been widely employed for chemical modifications due to its capability to quantify the redox behaviour of deposited nanomaterials in a triangular shape.

Microelectrode, a micro/nanoscale dimension of chemical sensors, has been widely developed as *in vivo* biosensing strategy to directly measure the analyte concentration inside the brain based on potential changes across chemically selective membranes at their tip.[167] Ding *et al.* developed an Au-microelectrode biosensor to detect Aβ level from CSF of live mice *in situ*.[225] They governed hemin and Cu^{2+} ion that typically bind to Aβ to induce strong coordination of Cu^{2+}, Aβ, and hemin. The deposited silver NPs onto Au-microelectrode respond to dynamic alterations of the Aβ, which is subsequently turned into an amplified selective signal. The CSF was sampled from cisterna magna of the mice vigilantly using microneedle to avoid the blood vessels damage. EIS and LSV methods were employed to characterize the probes-deposition and to quantify Aβ level, respectively. The detection limit was shown at 0.2 pM along with wide linear range from 1 pM to 50 nM. Similar concept of electrochemical application was extended using silk fibroin material by Liu *et al.* on the development of POC device for Aβ detection based on blood sample.[226] In addition, a thorough discussion on state-of-the-art of microelectrode-based *in vivo* neurochemicals sensing has been discussed in a review by Xu *et al.*[227]

A microelectrode principle was further employed by Peng *et al.* using carbon fibre to monitor the superoxide anion radical ($O_2^{\bullet-}$) which directly correlate with production of reactive oxygen species-induced Aβ.[224] They engineered ionic-liquid polymer with carbon nanotubes to mask the immobilized oxide dismutase (*i.e.*, catalysing $O_2^{\bullet-}$ into peroxide species) from the enzyme leakage thereby, achieving better sensitivity (Fig. 8). *In vivo* experiment was carried out by implanting the microelectrode into live rats' corpus striatum. The catalytic processes were examined using CV and amperometric methods. This study was able to exhibit as low as 0.42 μM of LOD with a decent linear relationship towards $O_2^{\bullet-}$ from 1.0 to 228.0 μM. The results also manifested the use of functionalized ionic liquid polymer (PIL) to preserve electrocatalytic activity, augment the stability, and serve as potential low-toxic matrix to support the substrate binding (*e.g.*, enzyme or probe).

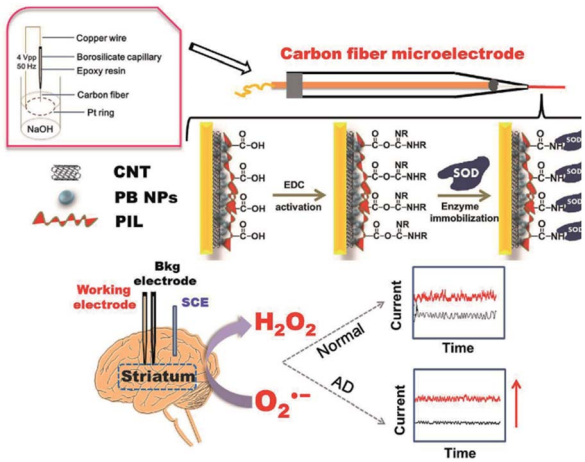

Fig. 8 Schematic illustration demonstrating the fabrication of superoxide dismutase/functionalized ionic liquid polymer/Prussian blue/carbon nanotubes/carbon fiber microelectrode (SOD/PIL/PB/CNT/CFME) sensor for the quantification of $O_2^{\bullet-}$. Reproduced from ref. 224 with permission. Copyright (2019) Elsevier.

For the *in vivo* biosensor, the need for miniaturized working electrode is important to minimize the possible adverse effect of tissue damage during implantation and to increase the spatial resolution towards probe discrete brain regions as well as the sampling rates.[218] Furthermore, translating the *in vivo* application into real human application remains another critical challenge. There are various biological aspects to be considered, which may differ in expected outcomes with the *in vivo*, such as uncontrolled inorganic material degradation of the implant in a complex biofluid. Other assessments are required, including systemic toxicity, immune response, irritation, that have satisfactorily been discussed by Gray and coworkers.[115]

3. A perspective on device's translational and commercialization pathways

Current development of biosensors has been paving the way on translating the sensing functionality to the personal healthcare device. Several types of biosensor products have successfully been distributed globally, including the blood-glucose, uric acid, cholesterol, and tropical disease diagnostics, which further improve the health core-facility down to suburban settings or the remote endemic. More importantly, the integrated application to non-invasively monitor the disease could facilitate better progress and plans for future medication. The presence of this particular technology should possibly never shirk the low cost and uncomplicated processing of a device. In accordance with this, the instrument-less biosensor, like a paper-based diagnostic tool, seems to be promising commercialization thereof.[228,229] Concurrently, there is a new trend in wearable sensors which have been reaching the public market since 2014 and expected to substantially increase by the year of progress.[229] Compared to the POC, this type of marketable sensor is predicted to supplement the market along with the highly needs for the updated smart device. However, several obstacles related to their capability prior to public commercialization need to be addressed. The instability of the device is the bottleneck that seems perpetual. The device must cope with the dynamic biofluid, entangled constituents present in the biofluid, and biorecognition capability during the period of use.[230–233] The robust transmission of the generated signal to translate the detection into readable or report-mode is also considered. The ideal wearable sensor may not be seen as yet, but the promising concept has been one-by-one adequately delivered and fenced with full delicacy to improve the personalized healthcare in the future.

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4. Conclusions and outlook

Designing suitable and reliable biosensor for AD have continually attracted great interests with the noteworthy growth in recent years. The key factors of these biosensor designs are based on the progress by corresponding biomarkers, sample sources, nanomaterials used as well as advanced transducing techniques. Particularly, it is worth to note that electrochemical biosensor is the most common technique to produce biosensor along with Aβ peptide and tau protein as frequent targets for diagnosing AD. The excellent sensitivity, easy-to-use, simple fabrication, and good selectivity which could be reached through this device architecture. Instead, the closeness and convenience to the end-users are other remarkable benefits of the electrochemical sensing inspired from the success story of glucose monitoring devices which are widely available as POC testing in market. On the other hand, plasmonic detection and fluorescent biosensor are also taken into account as promising strategies for diagnosing AD. Numerous types of other biosensors may serve as alternative methods towards POC diagnostics of the AD. Moreover, theoretical chemistry has admittedly validated and supported the experimental results by its central contributions towards determining the optimized geometry and binding motif of attributed molecular interactions (*i.e.*, *via* molecular dynamics and molecular docking).

Non-invasiveness of the sampling method and device sensitivity are the critical points in the successful development of biosensor for AD. Complying the POC diagnostics, further research could be conducted towards development of ultra-performance biosensors architecture by using biocompatible nanomaterials (*e.g.*, polyethylene glycol, chitosan, biopolymer, *etc.*), minimally-invasive samples (*e.g.*, blood, saliva), and attempting to reach single molecule analyte as ultimate goal of the device construction. However, from the physician viewpoint, the urgent need in current diagnostic device is not only devoted on the ultrasensitivity feature, but also the wide dynamic or linear range covering the clinical concentration of AD biomarkers. Molecular electronics and self-powered biosensors appear to be the future of bioelectronics. Currently, miniaturization of the system entering the mobile world (*i.e.*, biosensors based on mobile phone; Android or IOS) and self-powered device are vastly considerable in revealing advanced, smart, and early POC- and even beyond, eHealth-, based diagnosis platform of the disease.

Author contributions

B. T. M., A. D. P. developed the main conceptualization and wrote the original manuscript in consultation with Y. J. H., C. W. P., and P. K. Y. S. M. W., Y. J. H., C. W. P., and P. K. Y. assisted in writing, reviewing, and editing of the manuscript. All the authors contributed to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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