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Mini review

Electrochemical, Photoelectrochemical and Electrochemiluminescent Biosensors for the Detection of Beta-amyloid Peptides and Their Aggregates

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Beta-amyloid (Aβ) peptides are the specific biomarkers of Alzheimer's disease (AD). Many clinical studies have suggested that abnormal levels of Aβ peptides in blood, cerebrospinal fluid and brain tissue are closely related to the progression of AD. Analysis of Aβ peptides is of great significance to the early detection, prevention and treatment of AD. This paper focuses on the progress in development of electrochemical, photoelectrochemical and electrochemiluminescent biosensors for the detection of Aβ peptides and their aggregates.

Keywords: Beta-amyloid; electrochemical biosensors; photoelectrochemical biosensors; electrochemiluminescent biosensors; Alzheimer's disease

1. INTRODUCTION

Alzheimer's disease (AD) is an irreversible neurodegenerative disease. It is the most common format of dementia. The clinical manifestations of AD are memory, cognitive impairment and the decline of daily living ability. With the aging of the population, the incidence of AD is getting higher and higher. This not only endangers the health of the elderly, but also brings a heavy burden to the family and society. Thus, the disease has attracted wide attention. The cascade hypothesis of beta-amyloid (Aβ) peptide suggests that the formation of neurotoxic oligomers and fibroids during the

aggregation of Aβ is an important factor to induce AD [1]. Many clinical studies have suggested that abnormal levels of Aβ in blood, cerebrospinal fluid and brain tissue are closely related to the progression of AD, and thus Aβ has become one of the important biomarkers for AD [2]. Aβ is produced from enzymatic digestion of amyloid precursor protein (APP) by β- and γ-secretases [3-5]. The main species of Aβ peptides are $Aβ_{40}$ and $Aβ_{42}$, containing 40 and 42 amino acids, respectively. There are many forms of Aβ peptide in vivo, such as monomer, oligomer (AβO) and fiber (AβF). It has been suggested that the Aβ monomer has no neurotoxicity, but AβO and AβF formed by nucleation-dependent complex process exhibit neurotoxicity. Abnormal aggregation of Aβ is the pathological basis of AD. Thus, Aβ has important value in early diagnosis, prevention and treatment of AD. The dynamic instability of Aβ aggregation process makes it difficult to detect Aβ monomer or the aggregates. Therefore, it is necessary to establish sensitive and efficient analytical methods for detecting various forms of Aβ. In this paper, the methods of electronic detection of Aβ are summarized. Several electrochemical biosensors including electrochemical impedance spectroscopy, voltammetry, photoelectrochemistry (PEC) and electrochemiluminescence (ECL) are emphatically described.

2. ELECTROCHEMICAL BIOSENSORS

Electrochemical biosensor is a technique that uses biological active materials (such as enzymes, proteins/peptides, antibodies, microorganisms and nucleic acids) as the recognition elements to transform biochemical reactions into quantifiable physical/chemical signals for target detection [6, 7]. The technique has exhibited the most potential to determine Aβ because of its simple equipment, good selectivity, high sensitivity, fast response and low cost. The recognition elements for different forms of Aβ included antibodies, peptides, aptamers, gelsolin and so on. In this part, the electrochemical biosensors developed in recent years for the detection of Aβ peptides and their aggregates are reviewed according to the difference of recognition elements.

2.1. Direct electrochemical detection

The electrochemical biosensors without the use of any identification elements are simple and effective. The Tyr10 residue on the surface of Aβ is easily electrochemically oxidized. However, the aggregation of Aβ may cause its structural conformation change, which prevents the exposure of Tyr10 to the surface of Aβ. Based on this fact, the aggregation process of Aβ can be studied without labeling. For example, Vestergaard and co-workers first determined the changes of Tyr10 oxidation signals in the Aβ sequence with three electrochemical techniques: cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) with a glassy carbon electrode as the working electrode (Figure 1) [8]. The method can be used to study the aggregation process of $Aβ_{40}$ and $Aβ_{42}$. The results showed that the aggregation process of the two peptides was significantly different. Furthermore, Veloso and co-workers reported a SWV method for screening of Aβ aggregation inhibitors based on the reduction of Tyr10 oxidation signal on printed electrodes [9]. Suprun and co-workers found that the oxidation currents of Tyr10, three histidines (His6, His13 and His14) and

methionine (Met35) residues in Aβ decreased during the aggregation of $A\beta_{42}$ [10]. Enache and co-workers found that the electrochemical oxidation behavior of Aβ is dependent upon the length, hydrophobicity and 3D structure of the five amino acid residues in Aβ (Tyr10, His6, His13, His14, Met35) [11]. In addition, EI-Said and co-workers reported the CV detection of Aβ with electrodeposited AuNPs-modified indium tin oxide electrode [12]. The CV showed a characteristic oxidation peak at 0.8 V. The method was applied to detect the extract Aβ samples from AD-induced rat brains with a detection of limit (LOD) of 20.7 ng/g. The CV curves for the cases of both male and female rats showed apparent peaks at 0.8 V.

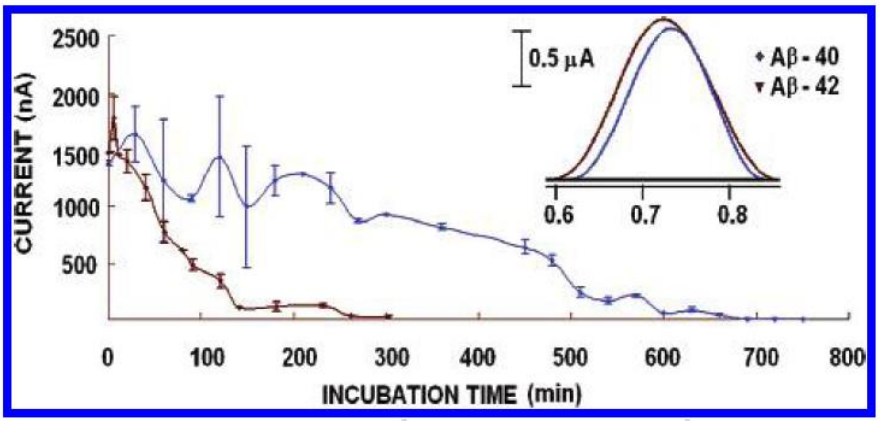

Figure 1. Kinetic study of Aβ-42 (red line) and Aβ-40 (blue line) aggregation after incubation at 80 íM in TBS at 37 ± 1 °C; detected at 8 and 4 µM, respectively, using SWV, at room temperature. Inset: Voltammograms of native Aβ-42 (red line) and Aβ-40 (blue line); detected at 8 and 4 µM, respectively, using SWV, at room temperature. Reprinted with permission from reference [8]. Copyright 2005 American Chemical Society.

2.2 Antibodies

Among kinds of receptors, antibodies are the most widely used recognition elements in designing of biosensors although efforts have made to replace antibodies with new biomolecules [13]. To data, the antibodies-based biosensors are still the most widespread devices for the detection of different forms of Aβ. Prabhulkar and co-workers preprared carbon fiber microelectrodes modified with three kinds of antibodies (mHJ2, mHJ7.4 and mHJ5.1) respectively [14]. Antibodies mHJ2 and mHJ7.4 recognized $A\beta_{40}$ and $A\beta_{42}$, respectively. Antibodies mHJ5.1 recognized $A\beta_{17\text{-}28}$, $A\beta_{40}$ and $A\beta_{42}$ as positive controls. Tyr10 oxidation current signals were quantitatively analyzed by SWV. The linear ranges of $A\beta_{40}$ and $A\beta_{42}$ were 20 ~ 50 nM and 20 ~ 140 nM, respectively. Li and co-workers constructed an immunosensor by immobilizing antibodies on magnetic nitrogen-doped graphene that can be magnetically adsorbed on the screen-printed gold electrode [15]. Potassium ferricyanide was used as the probe, and DPV was used to detect the current changes before and after the capture of $A\beta_{42}$. The quantitative analysis of $A\beta_{42}$ was carried out with a LOD of 5 pg/mL. Kerman's group developed two specific antibodies (A11 and OC) for AβO and AβF (Figure 2) [16]. The conformation-specific antibodies were used to detect AβO and AβF by covalently immobilizing them on the gold

electrode. EIS was used to quantify the level of the two forms of Aβ species with a fast response. The method was also used to evaluate the inhibition activity of two symtriazine-based inhibitors. Gold nanoparticles (AuNPs) are widely used in antibody-based Aβ electrochemical biosensors in view of their excellent photoelectric properties and biocompatibility for electrode modification and antibody labeling [17]. Wu and co-workers reported the EIS detection of $A\beta_{42}$ with AuNPs-deposited aluminium oxide film electrode for the immobilization of captured antibody 12F4 [18]. The linear range was 1 ~ 10 000 pg/mL, and the LOD was 0.01 pg/mL. Lien and co-workers established three kinds of impedance immunosensors for Aβ detection on printed chips [19]. The signals were generated because of the specific immune reactions between antibodies immobilized on the electrode and Aβ in solution. Various length of Aβ species were determined with the electrodes modified with different antibodies. The minimum LOD of this method was 0.57 nM. Carneiro et al. reported the direct detection of Aβ using gold electrode modified with mercaptopropionic acid (MPA) SAM and electrodeposited AuNPs [20]. The DE2B4 antibody specific to the amino acid region of $A\beta_{1\text{-}17}$ was assembled on the electrode surface for the target capture. The LOD was found to be 5.2 pg/mL by SWV and EIS. Zakaria and co-workers have developed label-free sensor platform for $A\beta_{40}$ detection using a disc-shaped platinum/iridium (Pt/Ir) microelectrode [21]. The Pt/Ir microelectrode was electropolymerized with poly (ortho-phenylenediamine), a conducting free amine-containing aromatic polymer. The LOD of the EIS immunosensor was 4.81 pg/mL with a linear range of 1 ~ 10^4 pg/mL. Dela Escosura-Muiz and co-workers sandwich-like electrochemical biosensor for the detection of Aβ using porous magnetic microspheres for the immobilization of capture antibodies, in which AuNPs modified with detect antibodies were used as signal markers [22]. The cathodic current of AuNPs in hydrochloric acid was measured by chronoamperometry (CA). The high porosity of porous magnetic microspheres can effectively increase the surface area and detection sensitivity. The LOD is as low as 19 pg/mL. The method was first applied to detect Aβ in the serum, plasma and cerebrospinal fluid of AD patients. No Aβ was detected in serum and plasma. The concentration of Aβ in cerebrospinal fluid was found to be about 811 pg/mL. The value was consistent with the result of ELISA. Liu's group demonstrated that 6E10 antibodies (specific to the common N-terminal of all Aβ fragments) immobilized on the 3-mercaptopropionic acid (MPA)-covered electrode can capture $A\beta_{1\text{-}16}$-heme-AuNPs conjugates [23]. The resulting nanocomposites on electrode surface can catalyze the reduction of O_2 to H_2O_2. Based on the competitive binding of target $A\beta_{40/42}$ and $A\beta_{1\text{-}16}$-heme-AuNPs with the 6E10 antibody, the catalytic current decreased. This method allowed for the detection of 10 pM $A\beta_{40/42}$. Han and co-workers prepared capture antibody-modified Au@Fc-Zn-MOF as the signal marker to detect Aβ [24]. AuNPs were modified on the surface of graphene and amino-terminal polyethylenediamine-covered electrode for the capture of Aβ. Finally, SWV was used to determine the concentration of Aβ in the range of 0.0001 ~ 100 ng/mL with a LOD of 0.03 pg/mL.

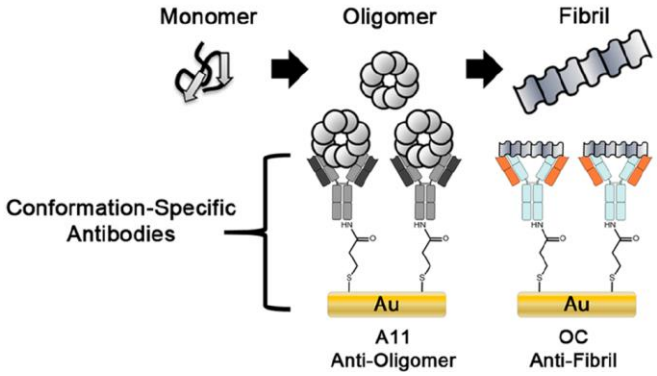

Figure 2. Detection principle for monitoring Aβ(1−42) fibrils and toxic oligomers using conformation specific antibodies in conjunction with EIS. Reprinted with permission from reference [16]. Copyright 2014 American Chemical Society.

Enzymes including alkaline phosphatase (ALP) and glucose oxidase (GOD) have been used in the detection of Aβ due to their high catalytic activity. Rama and co-workers reported the detection of Aβ using AuNPs-modified screen-printed electrodes for the immobilization of biotinylated Aβ [25]. When the mixed solution of target Aβ and its antibody was added, biotinylated Aβ and target Aβ competed to bind Aβ antibody. The attached antibody can capture IgG-conjugated ALP to catalyze the generation of electrochemical signal in the mixed solution of 3-indole-phosphate and Ag^+. The Aβ concentration range of this method was 0.5 ~ 500 ng/mL with a LOD of 0.1 ng/mL. In addition, Liu's group developed a competitive immunosensor to detect Aβ through the redox cycling of aminophenol [26]. Capture of biotinylated Aβ by the antibody-modified electrode facilitated the capture of the streptavidin-ALP conjugates. The captured ALP catalyzed the conversion of 4-aminophenol phosphate to p-aminophenol. In the presence of tri(2-carboxyethyl) phosphine (TCEP), p-aminophenol could be electrochemically oxidized on the electrode and then regenerated by TCEP to achieve the signal amplification. Because the target Aβ and biotinylated Aβ competitively bind to the immobilized antibody, the current decreased with the increase of the target Aβ concentration. The LOD of this method was 5 pM for both $Aβ_{40}$ and $Aβ_{42}$. Moreover, Diba and co-workers employed PEG, MPA and AuNPs-modified screen-printed carbon electrodes to construct a sandwich-like immunosensor for Aβ detection [27]. The capture and detection antibodies were antibody 12F4 and antibody 1E11 labeled with ALP, respectively. The specific catalytic reaction between ALP and p-aminophenol phosphate was monitored by DPV. The linear range was 100 fM ~ 25 pM, and the LOD was 100 fM.

The ratio of phosphorylated $Aβ_{40}$ ($pAβ_{40}$) to total $Aβ_{40}$ ($tAβ_{40}$) is expected to be used as an index for early diagnosis of AD. Very recently, Xiang's group reported the coimmunocapture and electrochemical detection of $pAβ_{40}$ and $tAβ_{40}$ (Figure 3) [28]. After simultaneous capture of $tAβ_{40}$ and $pAβ_{40}$ on the anti-$Aβ_{40}$-covered electrode, the level of $tAβ_{40}$ in cerebrospinal fluid samples was quantified by electrochemical method. With the signal amplification of Cd^{2+}/Ti^{4+}-functionalized titanium phosphate nanospheres (Cd^{2+}/Ti^{4+}@TiP) as the signal labels, the concentration of $pAβ_{40}$ was selectively determined by SWV. The LOD of this sensor was found to be 0.45 fM.

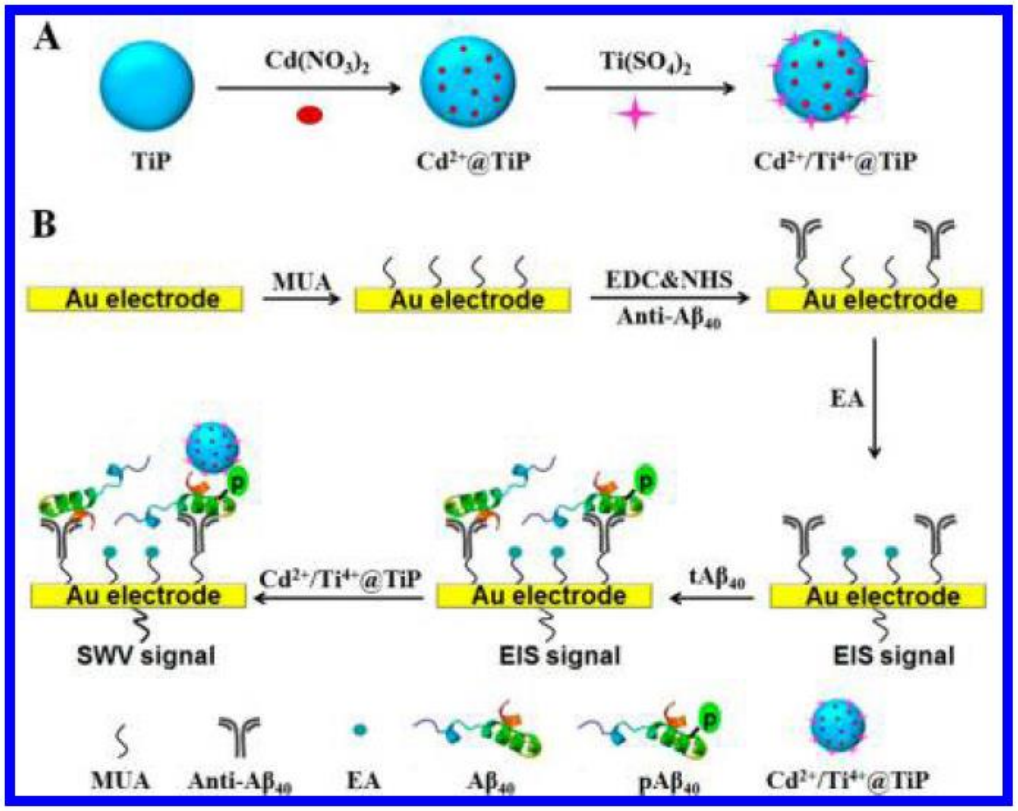

Figure 3. Schematic illustration of the electrochemical detection of tAβ40 and pAβ40. (A) Preparation of Cd^{2+}/Ti^{4+}@TiP. (B) Construction and detection principle of the electrochemical sensor. Reprinted with permission from reference [28]. Copyright 2014 American Chemical Society.

2.3. Peptides

There are few literatures on electrochemical biosensors for specific recognition of Aβ using specific peptides as the receptors. Beheshti and co-workers reported the detection of Aβ with four Fc-labeled peptide probes [29]. All the peptides included the Aβ hydrophobic sequence of KLVFF, but their C-terminals were modified with different groups. The interaction between Fc-labeled peptide and $Aβ_{12-28}$ immobilized on gold electrode surface was measured by CV and EIS. The results indicated that Fc-labeled peptide could prevent the formation of $Aβ_{12-28}$, depending on the nature of the modified group. Li's group reported the detection of AβO by assembly of functional peptide probe (RGTWEGKWK) labeled with 11-mercapto-alkanoic acid (MUA) and 9-mercapto-1-nonyl alcohol (MNH) on a gold electrode [30]. The method is based the hydrophobic interaction between peptide of RGTWEGKWK and the side chains of AβO. Because MUA is slightly longer than MNH, the peptide probes can swing or bend; thus, the binding of peptide probe with target AβO led to the change of electron transfer. This change was measured by SWV. AβO ranging from 0.48 to 12 nM can be determined with a LOD of 240 pM. Furthermore, Li's group also reported the detection of AβO based on the host–guest chemistry of cucurbituril (CB) (Figure 4) [31]. The electrochemical reporter of methyl viologen (MV) can be introduced into the surface of RGTFEGKF-MUA-modified electrode, thus generating electrochemical signals by forming CB/MV supramolecular complex. The binding of

AβO with RGTFEGKF inhibits the formation of the supramolecules by preventing the binding of MV onto the electrode surface. The current is inversely proportional to the level of AβO. The LOD of 48 pM is lower than that (240 pM) achieved by the above SWV method.

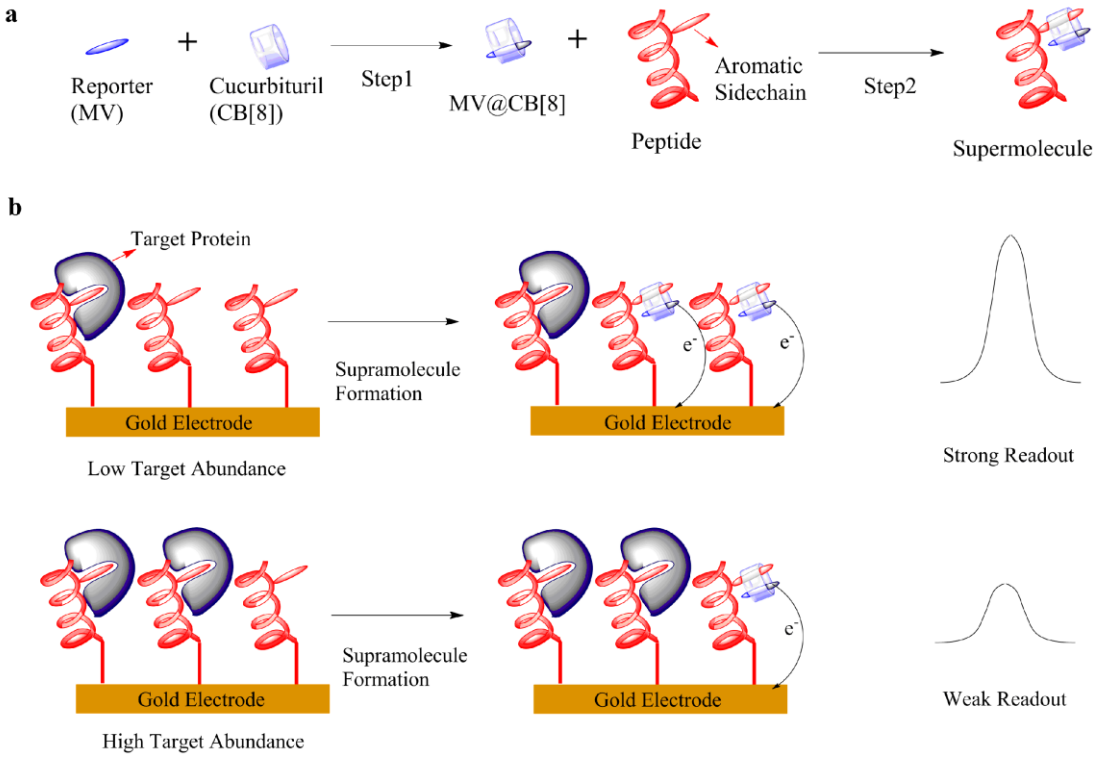

Figure 4. (a) Coupling peptide with reporter via supermolecule formation and (b) assay for protein detection. Reprinted with permission from reference [31]. Copyright 2013 American Chemical Society.

Cell prion protein (membrane-bound glycoprotein, PrP^C) has been identified as a high affinity receptor for AβO in genome-wide screening. The results showed that the core region for the interaction between PrP^C and AβO is $PrP_{95\text{-}110}$. These residues are located in the unstructured N-terminal region of PrP^C and have the amino acid sequence of THSQWNKPSKPKTNMK. This specific interaction between AβO and $PrP_{95\text{-}110}$ can allowed for the development of AβO biosensors [32-37]. For example, positively charged peptides can induce the assembly of negatively charged Au or Ag nanoparticles [37-39]; Liu's group reported the detection of AβO using adamantane (Ad)-labeled $PrP_{95\text{-}110}$ (Ad-$PrP_{95\text{-}110}$) as the signal probe and silver nanoparticle (AgNP) as the signal marker (Figure 5) [37]. Ad-$PrP_{95\text{-}110}$ caused the formation of a network of Ad-$PrP_{95\text{-}110}$/AgNPs by the assembly of AgNPs. The AgNPs-based network architectures were captured by the β-cyclodextrin (β-CD)-covered electrode through the host-guest interactions between Ad and β-CD. As a result, An amplified electrochemical signal from the solid-state Ag/AgCl reaction of AgNPs was observed. Once Ad-$PrP_{95\text{-}110}$ bound specifically with target AβO, the peptide would lose the ability to trigger the assembly of AgNPs and thus prevent the attachment of a network of Ad-$PrP_{95\text{-}110}$/AgNPs on the electrode surface. The current is inversely proportional to the level of AβO in the range of 20 pM ~ 100 nM. The biosensor showed a LOD as low as 8 pM and exhibited no response to Aβ monomer and AβF. Liu's group also constructed a sandwich-

like biosensor for AβO detection with the PrP_{95-110} probe [33]. ALP was used as the signal label and the signal was amplified by the electrochemical-chemical-chemical (ECC) redox cycling reaction with ferrocene methanol as the mediator. The detection range was 0.005 ~ 20 nM, and the LOD was 3 pM. Moreover, Rushworth and co-workers constructed an EIS biosensor for AβO detetion with biotinylated PrP_{95-110} as the probe [35].

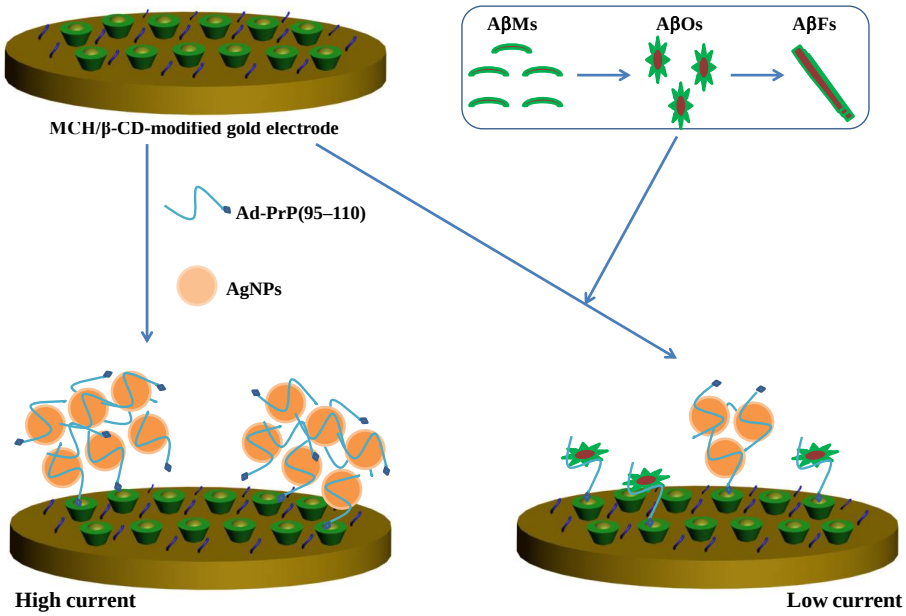

Figure 5. Schematic illustration of the electrochemical method for the selective detection of AβOs using AgNPs as the redox reporters and Ad-PrP(95–110) as the receptor. Reprinted with permission from reference [37]. Copyright 2016 American Chemical Society.

The probe was immobilized on the streptavidin-modified screen-printed gold electrode. Binding of AβO to the peptide probe induced the impedance change. The method was applied to detect the concentration of AβO secreted by 7PA2 cell in culture medium, and the concentration was found to be 24 nM. Recently, Qin and co-workers have developed an EIS biosensor for the selective detection of AβO with electrically conductive poly(pyrrole-2-carboxylic acid) (PPyCOOH) as the linking agent and PrP_{95-110} as the receptor [34]. The proposed biosensor has a low LOD of 10^{-4} pM.

2.4 Aptamers

Nucleic acid aptamers are oligonucleotide ligands with low cost, high affinity and specificity for target, simple structure and controlled-base sequence. In 2002, Ylera and co-workers found $Aβ_{40}$-specific aptamer by affinity column method [40]. In this work, cysteine-linked $Aβ_{40}$ was attached on the microspheres for the screening of aptamer. Lately, Takahashi and co-workers obtained RNA aptamers for $Aβ_{40}$ when screening the aptamers of α-synuclein oligomers [41]. They found that the aptamer can bind to both $Aβ_{40}$ and α-synuclein oligomers with high binding affinity. Thus, there are little reports for designing of $Aβ_{40}$ biosensors with the aptamer as the receptor in view of the poor specificity of the probe [42-44]. In 2016, Xu's group developed a "sandwich-like" immunosensor

using the Aβ antibody as the receptor and the aptamer as the recognition element (Figure 6) [43]. The signal was amplified with thionine-loaded AuNPs (Th-AuNPs) by measuring the reduction peak current of thionine. The feasibility of antibody-aptamer sandwich assay was confirmed by testing of AβO in artificial cerebrospinal fluid. Xu's group also developed an electrochemical AβO aptasensor using metal-organic frameworks (MOFs) as the signal probes [44]. The aptamer-tethered gold nanoflowers (AuNFs)-modified electrode was used for the capture of AβO. The aptamer-tagged gold nanoparticles/Cu-MOFs (AuNPs/Cu-MOFs) conjugates were attached on the electrode surface through the aptamer-AβO-aptamer interaction. The proposed aptasensor exhibited a linear range of 1 nM ~ 2 μM with a LOD of 0.45 nM. To date, the research in this field mainly focuses on Xu's group. The development of novel biosensors would be dependent upon the discovery of a variety of new Aβ or AβO specific aptamers.

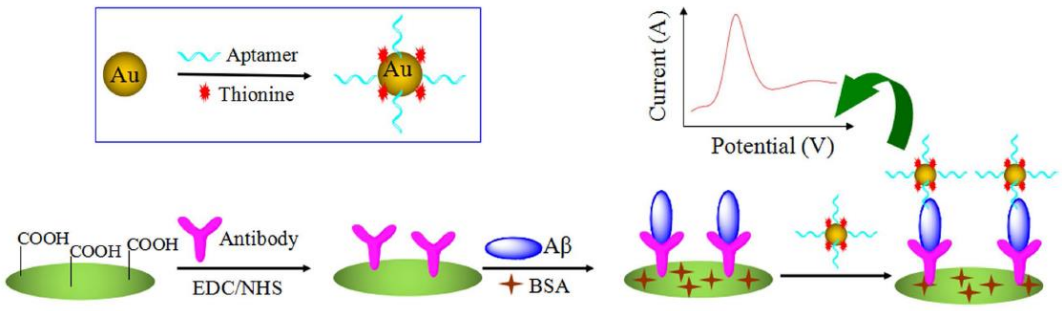

Figure 6. A schematic illustration of the electrochemical detection of A*β* oligomers using an antibody-aptamer sandwich assay. Reprinted with permission from [43]. Copyright 2016 Nature.

2.5 Other receptors

Gelsolin presented in plasma and cerebrospinal fluid is an important actin binding protein. It controls the structure of actin by cutting off, blocking actin filaments, or making actin aggregate and nucleate. In 1999, Chauhan and co-workers found that gelatin can selectively bind soluble Aβ in a concentration-dependent manner [45]. With gelsolin as the recognition element, Shi's group developed two sandwich-type electrochemical biosensors for the quantification of both $A\beta_{40}$ and $A\beta_{42}$ [46, 47]. In the first work, gelatin was coated on MWCNTs/AuNPs-modified electrodes for the capture of $A\beta_{40}$ and $A\beta_{42}$, and then the HRP-AuNPs-gelatin was conjugated with the captured Aβ species through the gelation-Aβ interaction (Figure 7) [46]. The electrochemical signal was recorded based on the electro-catalyzed oxidation of 3,3', 5,5'-tetramethylbiphenylamine (TMB) by HRP at 0.35 V. The method showed a detection range of 0.1 ~ 50 nM and a LOD as low as 28 pM. The complex was applied to detect Aβ in the cerebrospinal fluid from various brain regions of AD rats. In another work, the gelsolin-AuNPs-thionine bioconjugate was used instead of HRP-AuNPs-gelatin for the signal output [47]. The linear range was 0.2 ~ 40 nM, and the LOD was 50 pM. Besides these, there are few reports on the gelatin-based detection of Aβ because the pathway and mechanism for the gelation-Aβ

interaction need to be further investigated. Curcumin can depolymerize the Aβ aggregates through the curcumin-AβO interaction [48]. Curcumin or curcumin-Ni can be easily deposited on Ni foam by electrochemical polymerization. For this consideration, Qin and co-workers developed an EIS biosensor for AβO detection with curcumin as the receptor [49]. The curcumin-based sensor exhibited a proportional concentration relationship with AβO in the range of 0.001~ 5 nM. Moreover, the plastic antibody obtained by eletropolymerizing aniline (ANI) on electrode surface with $A\beta_{42}$ as the template has been demonstrated to determine $A\beta_{42}$ with a LOD of 0.4 pg/mL [50].

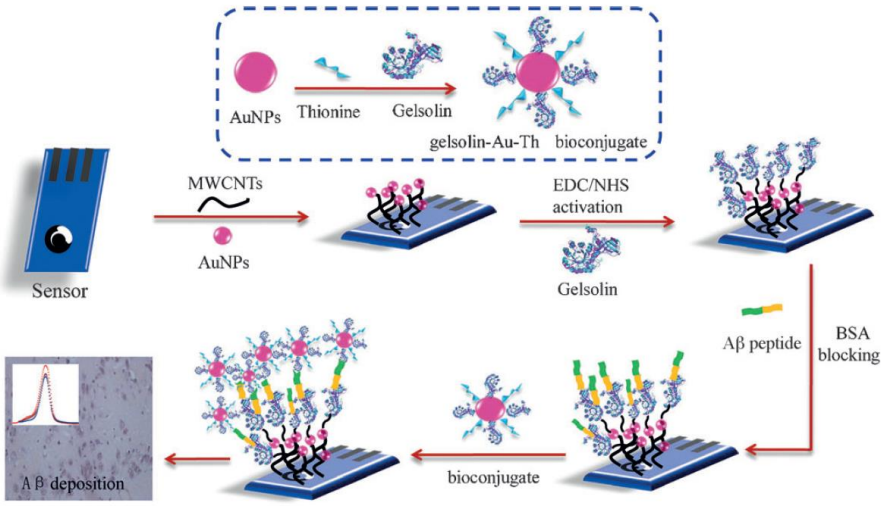

Figure 7. A schematic illustration of the electrochemical detection of Aβ(1–40/1–42) by using a gelsolin-Au-Th bioconjugate as a probe. Reprinted with permission from [46]. Copyright 2014 John Wiley and Sons.

3. PEC BIOSENSORS

PEC analysis is a new analytical method based on photoelectrochemical process and chemical/biological recognition process. This method employs light as excitation signal and photocurrent as detection signal. It has the advantages of high sensitivity, fast response, simple equipment and easy miniaturization. PEC sensor has attracted wide attention in the field of biological and environmental analysis. The photoelectric layer modified on the surface of the electrode is stimulated after absorbing photons, and the generated carriers are separated by charge and migrated by electron, resulting in production of photocurrent. Quantitative analysis of the target can be achieved by further modifying the sensing recognition unit on the photoelectric layer, using the quantitative relationship between the change of photocurrent caused by the direct redox, molecular recognition and binding, enzymatic catalysis and other reactions. Recently, PEC biosensors have been developed for Aβ detection [51-53]. For example, in view of the sensitization of Mn^{2+}-doped CdSe (Mn:CdSe) to Bi_2WO_6/CdS electrode, Zhang's group developed a PEC Aβ immunosensor. Aβ was labeled with Mn:CdSe [51]. The detection was carried out based on competitive binding of native Aβ and Aβ-Mn:CdSe to the antibody immobilized on the Bi_2WO_6/CdS electrode. The doped Mn^{2+} in CdSe caused the energy defect and hindered the recombination of photogenerated charge, thus greatly enhancing the

PEC response. The linear range varied from 0.2 pg/mL to 50 ng/mL with a LOD of 0.068 pg/mL. At the same time, Wei's group have developed two PEC immunosensors for Aβ detection based on $SnO_2/SnS_2/Ag_2S$ nanocomposites and $SnO_2/CdCO_3/CdS$ nanocomposites respectively [52, 53].

4. ECL BIOSENSORS

ECL immunoassay is a new generation of technology after radioimmunoassay, enzyme immunoassay, fluorescence immunoassay and chemiluminescence immunoassay. ECL is produced by the combination of chemiluminescent and electrochemical methods. It refers to a luminescence phenomenon that some special substances are produced by electrochemical methods, and then these electrogenerated substances react further with or between biomass and other substances. The technique retains the advantages of chemiluminescence such as high sensitivity, wide linear range, convenient observation and simple instrument.

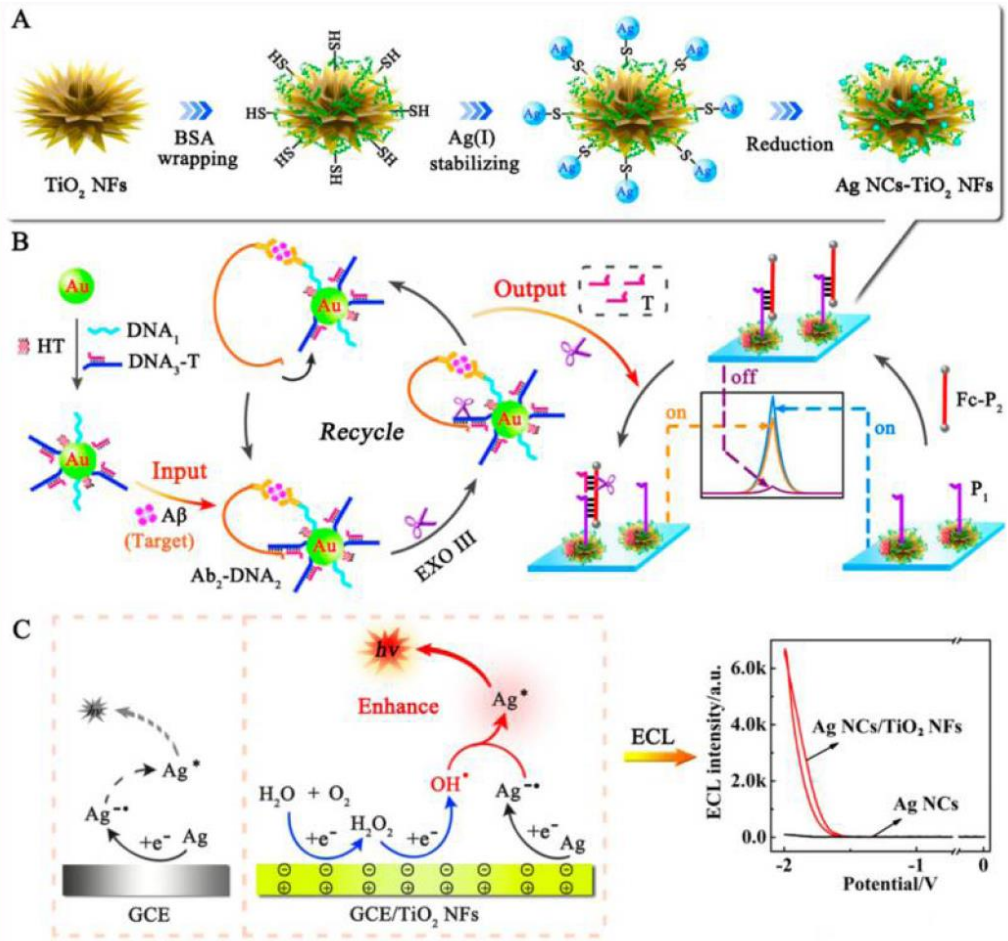

Figure 8. Schematic diagrams showing the preparation of Ag NCs-TiO_2 NFs (A), ultrasensitive detection of Aβ by combining immunoreaction-induced DNA nanostructure with Fc-driven light switch biodetection (B), and possible ECL emitting mechanism of Ag NCs-TiO_2 NFs (C). Reprinted with permission from reference [56]. Copyright 2016 American Chemical Society.

However, it has many advantages over the chemiluminescence method, such as good

reproducibility, stable reagents, easy control and reusability of some reagents. It has been widely used in biology, medicine, pharmacy, clinic, environment and food industry, immune and nucleic acid hybridization analysis and industrial analysis [54]. Recently, several ECL biosensors have been developed for Aβ detection.

For example, Yuan's group developed a luminal-based ECL biosensor for Aβ detection using flower-like nanomaterials doped with Ce:ZONFs [55]. Luminol was bonded to the surface of Ce:ZONFs by amidation and physical adsorption, and the complex of second antibody-GOD@Ce:ZONFs-luminol was used as a signal probe. The first antibody was fixed on the glassy carbon electrode modified by silver cysteine nanowires, and the second antibody complex was added in turn. GOD catalyzed glucose to produce hydrogen peroxide, Ce:ZONFs catalyzed hydrogen peroxide to produce free oxygen, and promoted luminol luminescence. Ce^{4+}/Ce^{3+} reaction can increase ECL reaction rate and signal through rapid electron transfer. The linear range of the method for Aβ is 80 fg/mL ~ 100 ng/mL, and the LOD is as low as 52 fg/mL. In view of the high ECL intensity of Ag NCs-TiO_2 NFs and high quenching efficiency of ferrocene (Fc), Yuan's group developed an ECL biosensor for quantitative detection of Aβ (Figure 8) [56]. Ag NCs-TiO_2 NFs were modified on the glassy carbon electrode to produce a strong ECL signal.

This case is called as the "switch on" state. The NFs acted as the active interface for the immobilization of thiolated capture DNA (P1). Once the DNA was triggered by the Fc-labeled assistant probes (P2) to hybridize with P1, the "switch on" state was translated into the "switch off" state due to the quenching ability of Fc towards Ag NCs. After that, the exposed part of P2 as an initiator hybridized with secondary-target DNA (T), triggering the Exo III cleavage reaction. This process was accompanied by the release of Fc, leading to the achievement of the second "switch on" state. A small amount of Aβ was then recognized by the immunoreaction-induced DNA nanostructure to output large numbers of secondary-target DNA probes. This could further trigger the release of Fc to recover the ECL signal. This method allowed for the ultrasensitive detection of Aβ with a wide linear range of 50 fg/mL~ 500 ng/mL and a LOD down to 32 fg/mL.

Moreover, Ke and co-workers developed a sandwich-like ECL biosensor for Aβ detection [57]. Firstly, magnetic epoxy-functionalized Fe_3O_4 nanoparticles were attached on magnetic glassy carbon electrode for immobilization of mesoporous carbon nanospheres@perfluorosulfonic acid/$Ru(bpy)_3^{2+}$/antibody. After incubation with Aβ, aptamer-modified gold nanorods were captured through the aptamer-Aβ interaction. The resonance energy transfer from $Ru(bpy)_3^{2+}$ to gold nanorods quenched the ECL signal of $Ru(bpy)_3^{2+}$.

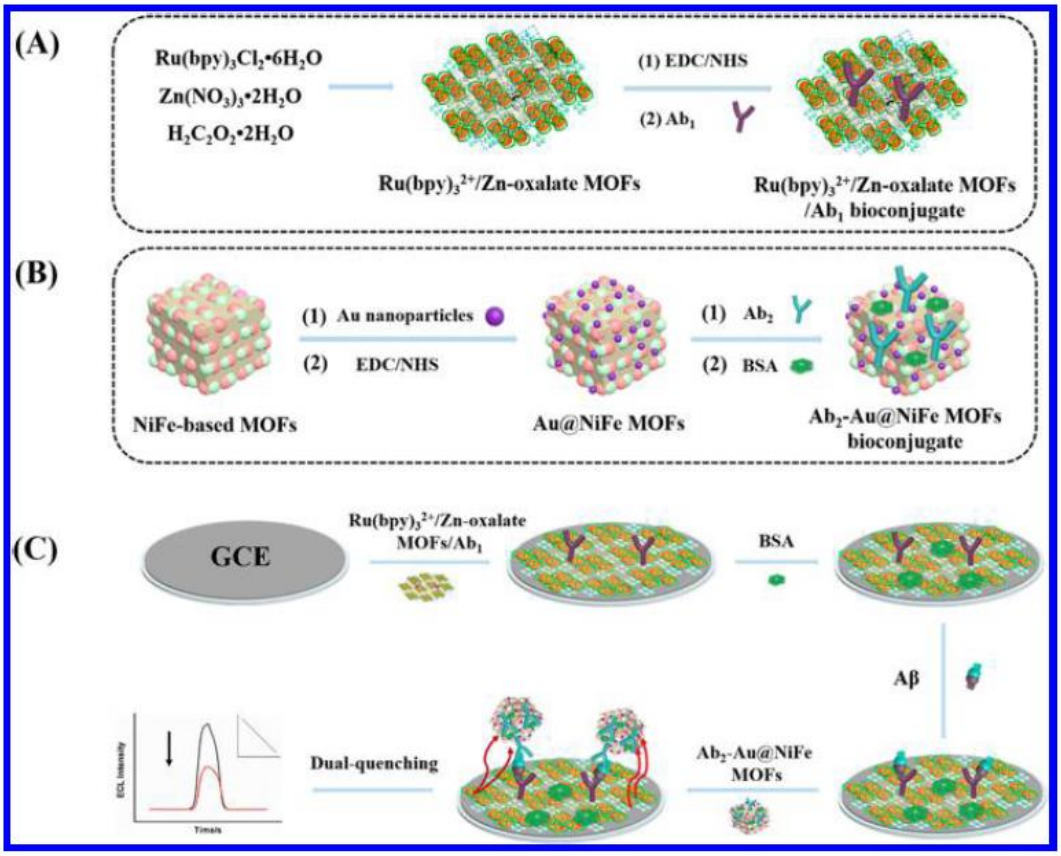

Figure 9. Preparation of $Ru(bpy)_3^{2+}$/zinc oxalate MOFs/Ab_1 bioconjugate (A) and Ab_2-Au@NiFe MOFs bioconjugate (B); construction process of the proposed dual-quenching ECL immunosensor (C). Reprinted with permission from reference [58]. Copyright 2016 American Chemical Society.

The linear range of this method for Aβ detection was 10^{-5} ~ 10^{2} ng/mL, and the LOD was 4.2 × 10^{-6} ng/mL. Very recently, Zhao and co-workers reported a double quenching ECL biosensor for Aβ detection with tris (2,2'-bipyridine)-ruthenium (II) [$Ru(bpy)_3^{2+}$] as the chromophore (Figure 9) [58]. [$Ru(bpy)_3^{2+}$] was immobilized in a three-dimensional (3D) zinc oxalate MOFs. The connectivity of three-dimensional chromophores in the MOFs provided a network for fast excited-state energy transfer between [$Ru(bpy)_3^{2+}$] units. The network prevented the release of chromophores to solution, thus resulting in high energy emission efficiency. Moreover, both AuNPs and NiFe-based nanocubic MOFs led to the reduction of ECL intensity of chromophores. The ECL emission spectra of three-dimensional [$Ru(bpy)_3^{2+}$]/zinc oxalate MOFs and the UV-VIS absorption spectra of Au@NiFe MOFs composites overlapped well, thus triggering the resonance energy transfer (RET) between [$Ru(bpy)_3^{2+}$]/zinc oxalate MOFs and Au@Nife MOFs to achieve double quenching effect. The linear range of the method is 100 fg/mL ~ 50 ng/mL and the LOD is 13.8 fg/mL.

5. CONCLUSION

This paper focuses on the development of electrochemical, PEC and ECL biosensors for the detection of Aβ and its aggregates. In view of the instability of Aβ in clinical samples, improving the

sensitivity, specificity and practicability of biosensors is still the goal of current research. From the reported investigations, the trends in development of electronic Aβ biosensors are as follows: First, the combination of nanomaterials and sensing technology can significantly improve the accuracy, sensitivity and response speed of analytical methods, thus providing new ideas and means for Aβ analysis. At present, the nanomaterials used in the analysis of Aβ are relatively limited, and there is still significant room to develop kinds of biocompatible nanomaterials. Second, the main biorecognition elements of Aβ are antibodies, peptides, gelatin, aptamers, and Aβ aggregation inhibitors. Each of the elements has its advantages and disadvantages. The combination of multiple recognition elements may improve the analytical merits. Third, Aβ in the single form, such as Aβ monomer or AβO, was analyzed in most reports. Simultaneous detection of different forms of Aβ species in a single electrode can reduce costs and analysis time and will be of importance for early detection, prevention and treatment of AD. Finally, integration of sensor design and sample pretreatment will improve the practicability of the sensors for clinical analysis.

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