Aβ Detection by Electrochemical Sensors

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Alzheimer's disease has taken the spotlight as a neurodegenerative disease which has caused crucial issues to both society and the economy. Specifically, aging populations in developed countries face an increasingly serious problem due to the increasing budget for patient care and an inadequate labor force, and therefore a solution is urgently needed. Diverse techniques for the detection of Alzheimer's biomarkers have been researched and developed to support early diagnosis and treatment. Among them, electrochemical biosensors and electrode modification proved their effectiveness in the detection of the Aβ biomarker at appropriately low concentrations for practice and point-of-care application.

Keywords: self-assembled monolayer ; Alzheimer's disease ; Aß detection ; electrochemical sensors

1. Electrochemical Sensors for Aβ Detection

Many studies have demonstrated that A β can be completely detected by employing an electrochemical sensor to obtain a low LOD at picograms or femtograms per milliliter ^{[1][2][3][4][5][6][7][8]}. The A β peptide structure has a tyrosine group, histidine, and methionine which participate in the redox reaction with an oxidized peak at ~0.6 and ~1.05 and wave at 1– 1.5 V vs. Ag/AgCl on a carbon surface ^{[9][10]}. The oxidation occurring on the residual groups under a supplied voltage indicates the detection ability for A β biomarkers; however, this method detects a plethora of other proteins containing such groups as well. Non-specific adsorption during the detection of such proteins could be eliminated by designing an electrochemical immunosensor in which non-specific adsorption is prevented by blocking the residual surface with bovine serum albumin (BSA) ^{[1][5][11]}.

The anti-A β antibody and A β antigen interaction taking place on the working electrode surface can be detected by diverse techniques such as CV ^[12], amperometry ^[13], DPV ^{[2][3][5][6][14][15]}, EIS ^{[1][2][16][17]}, and linear square voltammetry (LSV) ^[18]. Depending on the requirements, available equipment, and labor ability, each technique can be applied reasonably and effectively. Moreover, A β peptides can be detected using labels with enzymes, nanozymes, or anti-IgG-ALP, ^{[19][20]} or label-free using functional groups ^{[21][22][23]} in electrochemical detection methods.

2. Working Electrode Modification

The use of bare electrodes has shortcomings, such as low sensitivity, selectivity, and durability, which hamper the applicability of the A β electrochemical sensor due to a lack of an appropriate detection range and an inappropriate LOD, leading to it being less reliable and limiting the diagnostic application. To overcome these bottlenecks, many methods have been employed to modify the electrode surface, including designing different kinds of electrodes, synthesizing diversely structured nanomaterials, or amplifying the detectable signal. Among these, designing different kinds of electrodes requires expensive equipment along with skilled labor and amplifying the detectable signal is limited by a physical threshold, whereas diverse nanostructured materials on working electrodes have been intensively applied in laboratory environments employing metal, metal oxide, carbon, composite, conductive polymer, and SAM. Practically, tailoring the materials on the electrode surface to achieve a better performance has been conducted in a variety of research areas, like energy ^{[24][25]}, electrochemical sensors ^{[2][26][27]}, etc.

2.1. Metal, Alloy, and Metal Oxide

The noble metal Au has been documented to enhance the A β peptide detective performance of electrochemical sensors. Dai et al. deposited a thin film of Au on the working and counter electrodes by vapor deposition on the atomic level ^[15]. The SAM used 3-Mercaptopropionic acid (MPA), then EDC and NHS were added for functionalization to conjugate antibody A β 42. A β 42 was detected using the DPV technique with 5 mM [Fe(CN)₆]^{3-/4-} at various incubation times. The experiments were conducted in undiluted human serum at a linear range of 67.5–500 ng/mL. Lien et al. prepared a disposable electrochemical-printed (DEP) chip with Au deposition using CV techniques ^[16]. Then, antibodies were conjugated on the SAM layer to detect the A β peptides using the electrochemical impedance technique with 1–10³ nM

and an LOD of 2.65 nM. Further modification of the electrode with G protein increased the ability to detect $A\beta(1-42)$ with a linear range of 10 pM–100 nM and an LOD of 0.57 nM. Xia et al. reported an electrochemical immunosensor for the detection of A β oligomers (A β Os) ^[8], in which PrP(95-110) was labeled with adamantine (Ad) to form Ad-PrP(95-110), and then Ag nanoparticles (NPs) were add to a Ad-PrP(95-110) solution to form a mixture that was anchored onto a β -cyclodextrin (β -CD)-covered electrode surface through host–guest interaction; finally, the cyclodextrin-covered electrodes were incubated overnight with β -CD-SH and TCEP to link with the plate gold electrode, and the unreacted gold surface was blocked with the MCH solution. The fabricated sensor detected A β Os with a linear range of 20–100 nM and an LOD of 8 pM.

Metal oxide is inexpensive compared with novel metal and alloy, but it has proven to be effective in A β detection. Thus, the applicability of such materials in this kind of biosensor is immense.

2.2. Carbon-Based Materials

Carbon materials are famous for their high surface area, conductivity, and flexibility, which acts as a scaffold for tailoring or anchoring other elements in electrochemical biosensor applications. In addition, it contains many functional groups such as hydroxyl (-OH) and carboxyl (-COOH) which can be functionalized to conjugate antibodies for the antigen detection of AB. Sethi et al. designed a label-free electrochemical sensor based on the dual layer of graphene oxide and reduced graphene oxide to detect plasma-based A β_{1-42} ^[3], which was modified with 1-pyrenebutyric acid N-hydroxysuccinimide ester (Pyr-NHS) to assist in H31L21 antibody immobilization. As a result, $A\beta_{1-40}$ was recognized with a linear range of 11– 55 pM and an LOD of 2.398 pM. Chae et al. also constructed a carbon-based electrochemical sensor with the enhanced surface functionality of reduced graphene oxide (rGO) for diagnosing Alzheimer's disease [4]. GO was deposited on the substrate in 20 layers and was then treated with hydriodic acid (HI) to form a thin film of rGO, which was further treated to create a photo-resistant pattern, and finally lifted off; photolithography was then used to form the gold electrodes. The electrode was then incubated with EDC/NHS to immobilize 6E10 monoclonal antibody via covalent bonds with ethanolamine (ETA) to avoid any undesired covalent bonds. The oxygen-plasma-treated electrode exhibited a response 1.68-fold superior to that of the untreated electrodes in A β_{42} detection. Ji et al. investigated the effect of various materials functionalized on graphene for AB detection $\frac{[28]}{28}$ and recognized that the presence of AB on the surface of materials strongly affected electron transport. Hence, carbon-based materials are advantageous for fabricating electrochemical sensors for Aß peptide detection.

2.3. Composite Materials

Composite materials are widely utilized for electrochemical sensors in general and for electrochemical biosensors in particular thanks to the synergetic effect between diverse materials to enhance the electrical signal and, therefore, improvement in the sensitivity and stability. Zhou et al. prepared an electrochemical aptasensor of Au-deposited vertical graphene/carbon cloth (VG/CC), and then cellular prion protein (PrPC) residues 95-110 were immobilized on the electrochemical probes for the aptasensor. The fabricated aptasensor detected A β with a linear range of 10–2200 pM and an LOD of 3.5 pM. In another report, Li et al. prepared bifunctional Pd-decorated Co₉S₈ polysulfide nanoparticles supported on graphene oxide (G/Co₉S₈-Pd) ^[Z], which was deposited on the surface of GCE as a substrate for antibody conjugation by linking with Pd NPs after 1 h of incubation. The label-free electro immunosensor detected A β peptides with a linear range of 0.1 pg/mL–50 ng/mL and a low LOD of 41.4 pg/mL. Composite materials are promising candidates for electrochemical biosensor applications by improving the LOD toward higher sensitivity, which is a desired property for the design and practical application of biosensors.

2.4. Conductive Polymer

Conductive polymer with high electrical conductivity is also used for electrochemical sensors. In addition, functional groups on the structural surface facilitate the conjugation of antibodies for immunoreaction. Abbasi et al. prepared an electrochemical sensor with a conductive polymer with a controlled thickness on the screen-printed electrode ^[G]. Ultra-thin layers of polymerized 1,5-diaminonaphthalene (pDAN) were coated on the graphene layer of the electrode at a controlled thickness, and the anti-beta amyloid antibody was activated in a solution of EDC/NHS and conjugated on the conductive polymer; the free amine group was blocked by BSA. The sensor could detect $A\beta_{42}$ with a linear range of 1–1000 pg/mL, an LOD of 1.4 pg/mL, and a limit of quantification (LOQ) of 4.25 pg/mL. Zhao et al. prepared an electrochemical sensor with Au and PrP^c embedded in the conductive polymer matrix of poly(thiophene-3-acetic acid), poly(pyrrole-2-carboxylic acid), and poly (pyro-3-carboxylic acid) for the detection of A β O with a linear range of 10⁻⁹–10³ nM ^[29]. The PrP^c/AuNPs-E-Ppy-3-COOH-based sensor detected A β with an LOD of 10⁻⁹ nM. Conductive polymers with the advantageous features of high conductivity, flexibility, and structure-based functional groups are useful in electrochemical sensor applications.

2.5. SAM-Support-Based Working Electrodes for Aß Electrochemical Sensors

Self-assembled monolayer (SAM) is ordered arrays of organic molecules and has been employed as monolayers on electrode surfaces in liquid or solid phases in many electronic devices, such as electrochemical sensors [11][30][31], batteries ^[32], solar cells ^[33], and organic field effect transistors (OFETs) ^[34], through SAM modification ^[35]. The presence of an SAM on the working electrode structure acts as resistor, which contributes to the total impedance; thus, the study of SAM at a nanoscale level will help to tailor the chain length, create a defect-free electrode surface, and improve biosensor performance [36]. The layer orientation of the SAM tilts and twists at angles to the planar substrate surface, which varies with the material connected to the SAM; the presented atoms depend on the SAM composition with thickness-controlled layers [37]. A representative SAM structure consists of a head group (anchoring group), backbone (linkage), and tail group (functional group) [32][33][34]. The functionality of an SAM provides specific affinity to a substrate, while the alkanethiols on its structure enable adsorptability on the noble and coinage metals to form a high-order organic layer [38][39]. The thickness of an SAM is around 10 to 100 nm, and the deposition of an SAM on a metal surface can be performed by many techniques such as microcontact printing, scanning probes, and beams of photons, electrons, or atoms [11][30][35][37]. The SAM is deposited on a planar substrate that may be polycrystalline or single crystalline with a limited boundary [37]. Normally, Au- or Pd-based substrates are used for SAM [40][41] more frequently than other elements such as Ag-based substrates because of high conductivity, ease of bonding with the thiols group, avoidance of oxidation in the ambient environment, and nontoxicity to cells [42][43]. During SAM processing, ethanol has been used as a solvent because of its easy dissolution of alkanethiols, low cost, high purity, and low toxicity. The existence of terminally functional groups on the SAM surface allows for the immobilization of antibodies, enzymes, DNA, polypeptides, and proteins [34]. The ion-pair interaction on the SAM surface is also strongly affected by the pH range [44]. Both single and mixed SAMs can be used in micro/nano electronic devices owing to tunable SAM configurations, enhancement of stability, and modulation of the rectification properties, improving device performance [45]. To conclude, research on the biocompatible surface of SAMs facilitates electron transfer between electrodes and localized immunoreaction that contributes to the improvement of electrochemical sensors.

SAM construction on the working electrode can be carried out by diverse organic compounds, including organosulfur [37] ^[46], organosilanes ^[46], phosphates ^[47], and carboxylic acid ^{[46][48]}. Each of them required a type of substrate corresponding to head group structure. In the organosulfur case, thanks to the sulfur-containing group, Au-S chemical bonding formation can be easily achieved, facilitating this for the head group of alkanethiols anchoring on the Au surface by reductive elimination of the hydrogen. The bonding energy of the Au-S was estimated to be roughly 40 kcal·mol⁻¹ [25] $\frac{[32][34]}{2}$. Organosulfur-compounds-based SAM in electrochemical sensors has been employed for A β peptides detection $\frac{[1]}{2}$ [12][15][30]. In the case of organosilanes, silanol of the organic precursor can attach to hydroxylated surfaces, for example, silicon oxide (SiO₂), alumina, glass, zinc oxide (ZnO), indium oxide (In₂O₃), mica, and germanium dioxide (GeO₂) [34][46]. Generally, hydroxyl group is not available on such kinds of surfaces; therefore, the substrates should be treated by utilizing piranha solution or oxygen plasma to obtain required surfaces [34]. Generation of the -OH group after treatment makes the substrates hydrophilic, promoting the formation of a highly ordered monolayer. Compared to organosilanes and organosulfur compounds, which have been utilized frequently in fabricating SAM, whereas an insignificant amount of carboxylic-acid- and phosphate-based SAM was also studied on metal oxide substrates [46][48]. Based on the above analyses, the selection of SAM organic precursor strongly depends on the kinds of utilized substrates. Furthermore, SAM length, SAM concentration, SAM moiety, temperature, and incubation time decide the quality of the produced SAM, which therefore directly impacts SAM impedance. What is more, the type of moieties and their distribution determine the number of immobilized antibodies or enzymes, and these accompany biochemical reaction efficiency as well as sensor performance.

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