Nanomaterials in Neurodegenerative Disease Biomarkers

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The unique and outstanding properties of nanomaterials (such as graphene, carbon nanotubes, gold, silver and magnetic nanoparticles, polymers and quantum dots) have been contributing to enhance the electrochemical and optical behavior of transducers while offering a suitable matrix for the immobilization of biological recognition elements. Therefore, optical and electrochemical immuno- and DNA-biosensors with higher sensitivity, selectivity and longer stability have been reported. This review aims to provide insights into the conjugation of nanomaterials with different transducers highlighting their crucial role in the construction of biosensors for detection of Alzheimer's disease (AD) main biomarkers.

Biosensor Carbon nanotubes Graphene Gold Nanoparticles

In recent decades, novel modified transducers have been developed based on the unique properties of nanoscale materials and the ability to tailor their size and structure. As a result of quantum-size effects, nanomaterials exhibit great electronic, mechanical, thermal and optical properties and are recognized as one of the most attractive ways to promote the design of biosensors with enhanced analytical performance [1][2][3][4]. Integration of nanomaterials into biosensors has been found to improve the conductivity and catalytic activity of the transducer while favoring the immobilization of a large amount of biological recognition elements, as a result of their high surface area, in addition to improving the accessibility of specific analytes to these elements [2][3][4][5][6][7]. In fact, functionalization of nanomaterials has contributed to the development of highly sensitive and selective bioassays and biosensors for nucleic acids and proteins by integrating the biological recognition elements with the components of the various transduction mechanisms [5][7][8][9]. In this regard, considerable attention has been devoted to the immobilization of biological recognition elements as this aspect will have an impact on detection sensitivity, reproducibility and robustness, among other analytical parameters [4][8][10][11]. The performance of a biosensor is predominantly dependent on the binding affinity and specificity of binding molecules, their coating density onto the transducer’s surface and, finally and most importantly, the orientation of the biological recognition elements after immobilization, which should retain their full biological activity by ensuring that its binding sections remain intact and accessible while also providing an effective electronic connection between the redox active sites in the biomolecules and the transducer’s surface [3][5][12][13][14].

The most frequently applied nanomaterials in the development of immunosensors and DNA biosensors are carbon materials such as graphene and carbon nanotubes (CNTs), gold nanoparticles (AuNPs) and polymers, all having unique and specific properties applicable in the development of novel transduction schemes. Other nanomaterials such as silver (AgNPs) and magnetic nanoparticles, dendrimers and quantum dots (QDs) are also used, but to a

lesser extent. These nanomaterials have most commonly been reported for the development of biosensors performing detection via sandwich immunoassays/DNA assays or through direct detection (Figure 1).

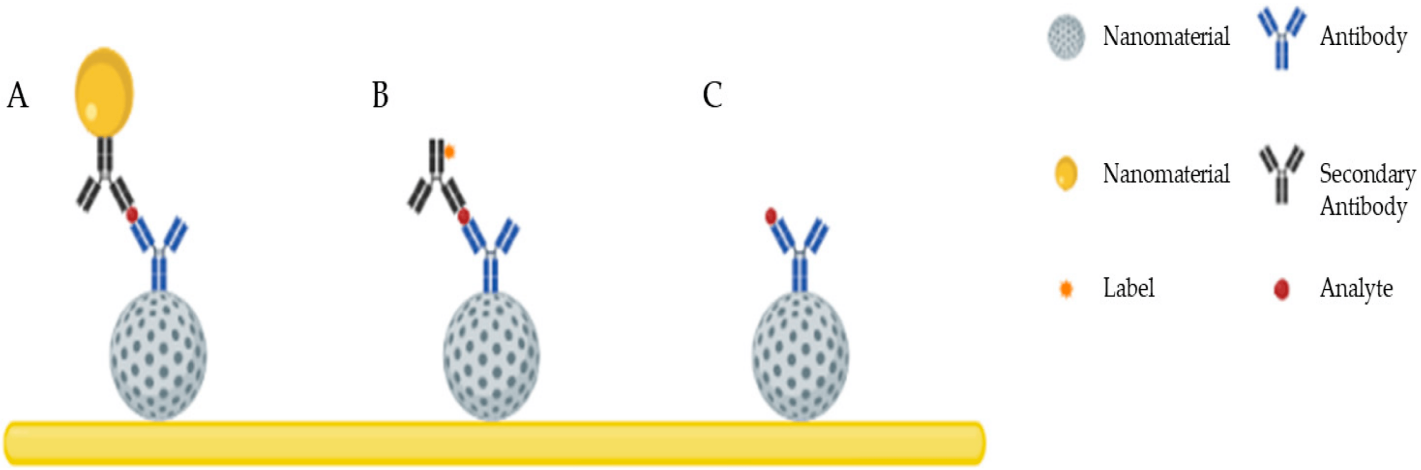

Figure 1. Schematic illustration of the most common detection schemes developed using nanomaterials: (**A**) sandwich assay with secondary antibodies conjugated with nanomaterials and without labels; (**B**) sandwich assay with labeled secondary antibodies; (**C**) direct detection.

Carbon Nanomaterials

Carbon-based materials including CNTs, graphene, fullerenes, carbon fibers, graphene quantum dots and carbon dots have been receiving a lot of attention in the development of biosensing analytical tools [12][15][16][17][18], with CNTs [19][20][21][22] and graphene [23][24][25] being the most commonly applied carbon nanomaterials for the development of biosensors for the detection of AD biomarkers. Although the specific characteristics of carbon nanomaterials vary between them, their most prominent advantages are reflected in their electrochemical activity, electrical conductivity, large surface area, high surface-to-volume ratio, ease of functionalization, biocompatibility and anti-fouling effect [8][4][9][12][15][17].

Carbon Nanotubes

CNTs are cylindrical large molecules consisting of a hexagonal arrangement of hybridized carbon atoms, which can be classified as single-walled carbon nanotubes (SWCNTs), composed of a single graphite sheet rolled into a seamless hollow nanoscale tube, and multi-walled carbon nanotubes (MWCNTs), characterized by the presence of multiple concentric tubes encircling one another [16][26][27]. SWCNTs present a diameter in the range of 0.4–2 nm, while MWCNTs, depending on the number of layers, can display a diameter in the range of 2–100 nm, with the distance between each layer being approximately 0.34 nm [4][5][16][27][28][29][30][31][32]. Since their discovery in 1991 [33], they have increasingly attracted research interest due to their high surface-to-volume ratio, exceptional electronic properties, and the presence of edge-plane-like defects which make them very interesting for biosensing applications [5][4][16][27][31][34]. In addition, another major advantage of CNTs is that they can be easily functionalized with different chemical groups through covalent and non-covalent bonds, which will further promote the

immobilization of biomolecules or organic molecules [4][5][12][31]. Four studies reported the use of CNTs for the development of biosensors towards determination of Alzheimer's disease (AD) main biomarkers. Oh et al. [19] developed a SWCNTs film-based biosensor with a metal semiconductor field effect transistor structure for determination of amyloid-beta 42 (Aβ42). In a different work Yu et al.[20] used SWCNTs to develop a ratiometric electrochemical biosensor for the simultaneous determination of Cu^{2+} and Aβ based on a glassy carbon electrode. For the determination of Aβ42/Aβ40 levels in cerebrospinal fluid and targeted brain tissue of AD rats, Yu et al. [21] developed an electrochemical affinity biosensor based on MWCNTs modified with AuNPs. In the last reported study using CNTs for biosensing of AD biomarkers, Lisi et al.[22] developed a biosensor for tau protein detection using a layer-by-layer approach for amplification of the surface plasmon resonance (SPR) signal.

Graphene

Graphene is a planar sheet of carbon atoms arranged into a rigid honeycomb structure, and like CNTs, the carbon bonds are sp^2-hybridized [35][36][37][38][39][40]. As a result of its electron configuration, graphene exhibits large surface area, high mechanical strength, high electrical conductivity, high elasticity and thermal conductivity [35][36][37][39][39][41]. Graphene oxide (GO) and reduced GO (rGO) are derivatives of graphene with a vast applicability in the biosensing field. Graphene can be easily functionalized into GO containing various oxygen functional groups, such as epoxide, carbonyl, carboxyl and hydroxyl groups [37][38][41]. These hydrophilic groups make GO more soluble in water, while exhibiting better selectivity towards functionalization with biomolecules, which are highly important features in biosensor applications [35][37][38]. On the other hand, rGO is the form of GO that is processed by chemical, thermal and other procedures which will ultimately influence its composition and properties [37]. The reduction process will reduce the oxygen content while introducing structural defects that will contribute to high thermal conductivity as the electrochemistry of graphene sheets occurs at the edges and defects (where heterogeneous electron transfer is fast) and not at the basal plane [36][37][38][41]. Usually, rGO presents advantages over graphene and GO for application in biosensing technology as it combines some of the negatively charged groups of GO along with the excellent conductive properties of graphene [40][42].

Graphene has been used in three different ways for the development of electrochemical immuno- and DNA biosensors for determination of AD biomarkers, namely, Aβ and apolipoprotein (ApoE), respectively. Two works performed determination of ApoE through DNA detection, with Mars et al.[23] using graphene quantum dots for a dual biosensing platform with electrochemical and fluorescence determination, while Wu et al. [24] conjugated graphene with mesoporous silica for the development of a hybrid nanomaterial that served as the basis for ratiometric determination. Finally, Kurkina et al.[25] applied rGO for the development of a FET electrochemical immunosensor for Aβ determination.

Nanoparticles

Nanoparticles with varied configurations and properties have been the most frequently used nanomaterials for the development of novel immuno- and DNA-biosensors [43][44]. Owing to their small size and high surface area, nanoparticles offer unique chemical, physical and electronic properties that are advantageous for the development

of high-performance biosensors either by using them as amplifying labels for signal enhancement or by working as an appropriate platform for the immobilization of biological recognition elements. The most common applied nanoparticles are metal nanoparticles, which are typically synthesized by chemical reduction of the corresponding transition metal salts in the presence of a stabilizer promoting the synthesis of nanoparticles with high stability, rich linking chemistry and solubility [43][44][45][46][47].

Gold Nanoparticles

Gold nanoparticles (AuNPs) are one of the most frequently applied nanomaterials in the biosensing field due to their inherent characteristics such as extraordinary optical and electronic properties, controllable morphology and size, and simple preparation methods [48][49]. In addition, AuNPs exhibit high chemical stability, excellent conducting capability and catalytic activity, large surface area and high surface-to-volume ratio, biological compatibility, and ease of functionalization, favoring the immobilization of biological recognition elements as DNA, antibodies and enzymes [3][8][9][12][48]. These features make AuNPs an outstanding nanomaterial for bridging the biological elements with the transduction systems [49].

AuNPs have been extensively used for the development of immuno- and DNA-biosensors for detection of AD biomarkers, being the nanomaterial more frequently reported throughout the reviewed studies. Being very versatile, AuNPs have been applied with different and complementary functions, such as promotion of the conductivity and electrochemical activity of the transducer, immobilization of the biological recognition elements for direct/sandwich detection or conjugated with other nanomaterials. The most common applications of AuNPs were with self-assembled monolayers (SAM), either by being deposited on a SAM-modified platform or serving as the platform for promoting the chemical modification with different SAMs [50][51][52][53], and as amplifying agents modified with secondary antibodies and labels for sandwich immunoassays [21][54][55][56][57]. Finally, AuNPs have also been applied in an isolated manner to transducer surfaces [58][59][60] or in conjugation with other materials such as polyethylene glycol (PEG) [61], MWCNTs [21], and ionic liquid [62]. The published studies clearly acknowledge the vast scope of AuNPs application.

Moreover, other nanomaterials such as AgNPs and magnetic nanoparticles, QDs, natural and synthetic polymers have also been used for the development of high-performance biosensors with varied configurations.

The publication can be found here: https://www.mdpi.com/2079-4991/9/12/1663/htm

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