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Nucleic acid-based electrochemical nanobiosensors

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ABSTRACT

The detection of biomarkers using sensitive and selective analytical devices is critically important for the early stage diagnosis and treatment of diseases. The synergy between the high specificity of nucleic acid recognition units and the great sensitivity of electrochemical signal transductions has already shown promise for the development of efficient biosensing platforms. Yet nucleic-acid based electrochemical biosensors often rely on target amplification strategies (e.g., polymerase chain reactions) to detect analytes at clinically relevant concentration ranges. The complexity and time-consuming nature of these amplification methods impede moving nucleic acid-based electrochemical biosensors from laboratory-based to point-of-care test settings. Fortunately, advancements in nanotechnology have provided growing evidence that the recruitment of nanoscaled materials and structures can enhance the biosensing performance (particularly in terms of sensitivity and response time) to the level suitable for use in point-of-care diagnostic tools. This Review highlights the significant progress in the field of nucleic acid-based electrochemical nanobiosensing with the focus on the works published during the last five years.

1. Introduction

Analytical devices enabling fast and cost-effective analysis of clinical samples are of growing demands for the early stage diagnosis and management of diseases. In this context, biosensors with electrochemical signal transductions hold great promise for the detection of trace levels of clinically relevant analytes. In comparison with mechanical- or optical-based sensing devices, electrochemical biosensors offer the advantages of low cost, simplicity, and the capability of being miniaturized. When integrated into lab-on-a-chip devices, electrochemical biosensors provide the possibility of analyzing small samples within short time frames, which is particularly useful for personalized therapy.

Centralized to any biosensing platform is biomolecular recognition. The development in biology over the past decades has proved that nucleic acids are not only hereditary materials for encoding genetic information but also ideal candidates for recognizing a variety of bioanalytes: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) can be easily detected through base-pairing with complementary nucleic acid probes, and non-nucleic acid targets can be specifically recognized by employing ligand-binding DNA or RNA sequences (aptamers). DNA tetrahedra, DNAzymes, and other functional DNA assemblies have further increased the attractiveness of nucleic acids for use in the design of electrochemical biosensors.

During the past 15 years, the field of nucleic acid-based electrochemical biosensing has witnessed great progress, thanks to the merging of biosensing research area with nanotechnology. Unprecedented biosensing opportunities have been achieved by employing nanomaterials with unique physicochemical properties in different aspects of electrochemical detection schemes. This has consequently propelled nanotechnology-based detection methods to the forefront of the biosensing research field. In the present Review, we highlight recent advances in the field of nucleic acid-based electrochemical nanobiosensors. We have itemized different classes of nanoscaled materials and structures that have been used in nucleic acid-based electrochemical biosensors and explained how they can contribute to the enhanced performance of such biosensors.

2. Nanoparticles

2.1. As electrode materials

Noble metal nanoparticles (NPs), with excellent conductivity, chemical inertness, and biocompatibility, have been extensively explored for improving the performance of nucleic acid-based electrochemical biosensors. As electrode modifiers, metal NPs can not only provide

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large active surface area but also facilitate the electron transfer processes at the sensing interface, thereby imparting the biosensors high sensitivity (Xiang et al., 2017; Zhao et al., 2014). In the cases where metal NPs are employed as anchoring sites for probe molecules, the controlled immobilization and the suitable orientation of the capture probes on the electrode-supported NP islands may allow efficient biorecognition, which further improves the sensing sensitivity (de Oliveira Marques et al., 2009; Li et al., 2005). As a result of these unique properties, metal NPs-modified sensing interfaces have been fabricated and used for the electrochemical detection of a wide range of biologically relevant targets, including nucleic acids (M. Wang et al., 2014), proteins (Su et al., 2016a), cells (Chandra et al., 2013), amino acids (Liang et al., 2011), and pathogens (Chand and Neethirajan, 2017), among others. NPs of metal oxides such as IrO_2 (Mayorga-Martinez et al., 2015), TiO_2 (M. Wang et al., 2015), and ZrO_2 (Hu et al., 2011) have also been employed as electrode modifiers in nucleic acid-based electrochemical biosensors.

2.2. As redox tracers

Metal NPs can serve as tracers in electrochemical biosensors based on their redox properties. In the method developed by X. Li et al. (2015), a target DNA linked an Ag NP with a magnetic bead through base-pairing with partially complementary DNA strands attached to both particles. The formed sandwich complex was then injected into a cost-effective and user-friendly paper-based electrochemical device for DNA analysis. Based on the electrochemical signal of silver, they quantified a nucleotide sequence characteristic of DNA from hepatitis B virus (HBV) with a limit of detection (LOD) of 85 pM, which was not low enough for early detection of HBV. Measurements with higher sensitivity have been carried out by employing aggregates of NPs. As reported by Li et al. (2010), the use of Ag NP aggregate tags, which were ten times larger than the individual NPs, yielded a strong electrochemical signal that offered the possibility of detecting a HBV-related DNA at a concentration as low as 5 aM.

More recently, Song et al. (2014) realized multiplex protein analysis by employing two kinds of nucleic acid-modified Ag NP tags, each of which functionalized with aptamers specific to platelet-derived growth factor (PDGF-BB) and thrombin as well as DNA strands complementary to those on the other NP (Fig. 1). They used a disposable electrochemical array composing of four working electrodes of W1, W2, W3, and W4, which were functionalized with PDGF-BB-specific aptamers, thrombin-specific aptamers, a mixture of both, and irrelevant single-stranded DNAs (ssDNAs) as control, respectively. The presence of target mediated the formation of sandwich complexes between the relevant electrode-immobilized aptamers and the functionalized Ag NPs, which was followed by hybridization-induced formation of Ag NP aggregates. Sensitive analysis of the proteins was then performed by stripping voltammetric detection of the silver ions released from the Ag NP aggregates through a pre-oxidation step. Other than NP aggregates, the deposition of silver on Au NP tags (Zhu et al., 2013) or the use of NPs–loaded carriers (Zhu et al., 2012) was demonstrated to improve the detection sensitivity significantly. Also, biosensors based on the in situ DNA-templated syntheses of metal NPs and nanoclusters (NCs) have shown remarkable sensitivity toward nucleic acid and protein analysis (Y. Wang et al., 2017; Yang et al., 2015; Zhu et al., 2016).

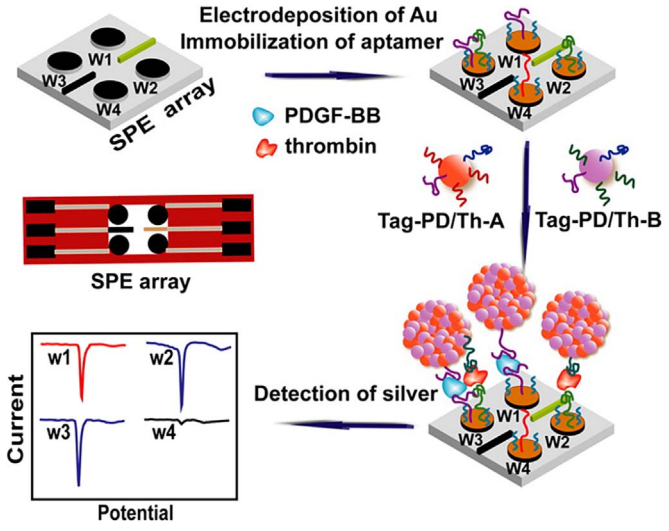

Fig. 1. Schematic representation of the electrochemical aptasensor for multiplex detection of PDGF-BB and thrombin. Reprinted with permission from Song et al. (2014). Copyright 2014 American Chemical Society.

2.3. As catalytic labels

Analogous to enzyme labels, some metal NPs can translate biorecognition events into significantly amplified signals by catalyzing a specific chemical reaction. The use of metal NP catalysts instead of enzymes in biosensing is beneficial as it allows overcoming the limitations associated with the poor thermal and environmental stability of enzymes. Moreover, in contrast to enzymes with only a small number of active sites (often just one), NP catalysts possess numerous active sites on their surface. As a result, when used as catalytic labels, NPs induce the generation of larger electrocatalytic signals than enzymes, thereby offering higher detection sensitivity. A typical detection scheme exploiting NPs catalytic properties for signal amplification relies on the target-mediated formation of a sandwich complex between a metal NP-linked reporter probe and an electrode-immobilized capture probe. The captured NP then causes the generation of a strong electrochemical signal by catalyzing the oxidation or reduction of a substrate, which can be used for the amplified detection of biomolecular targets (Hun et al., 2015; Polsky et al., 2006).

Other strategies relying on signal amplifications by NP-based catalytic labels have also been reported (Castañeda et al., 2017; de la Escosura-Muñiz et al., 2016; Li et al., 2012). Li et al. (2012) developed a reagentless method for hepatitis C virus (HCV) analysis by exploiting the hybridization-induced structural switching of a hairpin DNA probe immobilized on an electrode surface and conjugated to an Au NP at its free end. In the absence of the target DNA related to HCV genotype 1b, the stem-loop structure of the hairpin DNA kept the Au NP close to the electrode surface, allowing for efficient electron transfer with the electrode and enabling the electrocatalytic reduction of dissolved O_2. Upon hybridization, the stem-loop structure opened and the formed double-stranded DNA (dsDNA) moved the Au NP away from the electrode surface where the electron transfer was suppressed owing to the large distance. In a different methodology, de la Escosura-Muñiz et al. (2016) performed isothermal amplification of *Leishmania* DNA using primers labeled with magnetic beads and Au NPs. They magnetically collected the double-labeled DNA products on a screen-printed carbon electrode where the electrocatalytic activity of the Au NP tags toward the hydrogen evolution reaction allowed for detecting as low as 0.8 parasites per mL of blood (8×10^{-3} parasites per DNA amplification reaction).

In situ DNA-templated synthesis of metal NCs with electrocatalytic activities toward specific chemical reactions has recently been considered as an effective route for the amplified transduction of biorecognition events without the need for DNA functionalization of NCs. In this context, Z. Wang et al. (2015) developed a reusable microRNA (miRNA) sensor by taking advantage of the DNA/RNA heteroduplex-templated formation of Cu NCs and the electrocatalytic properties of these NCs toward hydrogen peroxide (H_2O_2) reduction. The sensor permitted sensitive quantification of target miRNAs with a LOD of 8.2 fM. In another study, Chen et al. (2015) combined target binding-triggered hybridization chain reactions (HCR) with the peroxidase-like activity of Ag NCs formed on cytosine-rich parts of the HCR-produced