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Carbon nanostructures as immobilization platform for DNA: A review on current progress in electrochemical DNA sensors

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ABSTRACT

Development of a sensitive, specific and cost-effective DNA detection method is motivated by increasing demand for the early stage diagnosis of genetic diseases. Recent developments in the design and fabrication of efficient sensor platforms based on nanostructures make the highly sensitive sensors which could indicate very low detection limit to the level of few molecules, a realistic possibility. Electrochemical detection methods are widely used in DNA diagnostics as it provide simple, accurate and inexpensive platform for DNA detection. In addition, the electrochemical DNA sensors provide direct electronic signal without the use of expensive signal transduction equipment and facilitates the immobilization of single stranded DNA (ssDNA) probe sequences on a wide variety of electrode substrates. It has been found that a range of nanomaterials such as metal nanoparticles (MNPs), carbon based nanomaterials, quantum dots (QDs), magnetic nanoparticles and polymeric NPs have been introduced in the sensor design to enhance the sensing performance of electrochemical DNA sensor. In this review, we discuss recent progress in the design and fabrication of efficient electrochemical genosensors based on carbon nanostructures such as carbon nanotubes, graphene, graphene oxide and nanodiamonds.

1. Introduction

DNA biosensors are analytical devices fabricated by the integration of a sequence-specific DNA probe and a transducer (Lucarelli et al., 2008; Palecek and Jelen, 2002). At present, the development of a sensitive, specific and cost-effective DNA detection method is in high demand for the diagnosis of genetic diseases. Electrochemical detection methods are one of the widely used analytical methods in DNA diagnostics as it provide simple, accurate and inexpensive platform for DNA detection. In addition, the electrochemical DNA sensors provide direct electronic signal without the use of expensive signal transduction equipments (Grabowska et al., 2014; Liu et al., 2008; Wang, 2006). Another advantage of electrochemical DNA sensing is the availability of a wide variety of electrode substrates and various modification strategies of the electrode which pave the way for efficient immobilization of the single stranded DNA (ssDNA) probe sequences (Feng et al., 2011; Garcia-Mendiola et al., 2013; Jayakumar et al., 2012; Li et al., 2012a).

A range of nanomaterials such as metal nanoparticles, quantum dots, magnetic nanoparticles and polymeric NPs have been introduced as electrochemical labels in the sensor design to enhance the sensing performance of electrochemical DNA sensors (Gill et al., 2008; Holzinger et al., 2014; Kerman et al., 2008; Kurkina et al., 2011; Wu et al., 2014; Zhu et al., 2015). The substrate used for the immobilization of DNA strands also greatly influence the response of the electrochemical DNA sensor. Nanomaterials such as gold nanoparticles (AuNPs), carbon nanotubes (CNTs), composites of carbon nanotubes, graphene, graphene derivatives, doped graphene derivatives and its nanocomposites in conjunction with oxide nanoparticles and polymeric carbon nitride have been used for modification of these electrodes to improve the sensitivity and stability of the sensor (Cao et al., 2011; Chen et al., 2012; Gao et al., 2014; Jacobs et al., 2010; Kuila et al., 2011; Saha et al., 2012; Shao et al., 2010; Wang, 2012). Carbon nanostructures showed great promise as immobilization platform in electrochemical DNA sensors due to its characteristic properties like fast electron transportation, high thermal conductivity, excellent mechanical flexibility, rapid electrode kinetics, ease of functionalization and large surface area (Kato and Niwa, 2013). The extraordinary electrochemical characteristics of carbon nanotube and graphene and its derivatives make them ideal for Faradaic processes and non-Faradaic processes. These carbon structures can accept or withdraw electrons from or to molecules adsorbed on them leading to large changes in conductance which can easily be detected. These features make them as promising materials for electrochemical biosensing.

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However, the presence of any charged ions other than analyte within the Debye length around these carbon nanostructures are prone to produce the same kind of charge transfer effect, which may affect the selectivity of the sensor due to the interference of the charged molecules/ions. Similarly, due to the various kinds of interactions possible between biomolecule and the carbon nanostructures, there is a possibility of non-specific binding, which must be avoided before the sensing experiments. This can be overcome by using membranes or functional layers. Mainly the disadvantages of the CNTs, graphene and other carbon nanostructures are due to the inhomogeneity in structure and size. This can lead to less repeatability and uncertainty in the measurements. The electrode performance of these nanostructures depend on their synthesis method, method of functionalization, method of electrode attachment and addition of mediators. Significant efforts have been invested in recent years to overcome these drawbacks as evidenced by the numerous contributions that appear in literature. This review highlights the various fabrication method of the electrodes based on carbon nanostructures such as CNTs, Graphene, nanodiamond and their composites for the sensitive and selective detection of target DNA.

2. Carbon nanostructures as immobilization platform in DNA sensors

The carbon nanostructures commonly used in DNA sensors are single walled carbon nanotubes (SWCNT) or multi walled carbon nanotubes (MWCNT), graphene oxide (GO), reduced graphene oxide (rGO), graphene (Gao et al., 2014; Jacobs et al., 2010; Kuila et al., 2011) and nanodiamonds (Yang et al., 2008). DNA bases interact with graphene or CNT surfaces through van der Waals interaction (π interaction) and solvation energy provided by the solvent molecules (Fu and Li, 2010). The interaction of DNA with carbon surfaces or the binding of two DNAs on the surface may result in significant changes in the electrochemical signal and this mechanism is used for sensing DNA with high sensitivity and single base specificity (Lv et al., 2010; Tang et al., 2006). The use of each of these immobilization platforms are described in detail in the following sections.

2.1. Carbon nanotubes

Carbon nanotubes (CNT) are built from sp^2 carbon units with hexagonal honeycomb lattices in several nanometers in diameter and many microns in length (Rivas et al., 2007). Although CNTs are available in different forms, SWCNTs and MWCNTs are the most commonly used sensing surfaces for electrochemical applications. In a transducer, CNTs act as a part of the transducer and as a carrier for DNA probes. In addition, it promotes electron transfer between electroactive species and electrodes. Therefore, CNTs have been widely used for modification of electrode to design electrochemical sensing platform (Yang et al., 2007). CNTs can be easily functionalized with different chemical groups (Balasubramanian and Burghard, 2005; Zhao and Stoddart, 2009) by both covalent or non-covalent procedures and functionalized CNTs can be conjugated to a variety of biomolecules for different bioanalytical applications. Non-covalent functionalization of CNTs is important to preserve the structure and its electronic characteristics. The CNT based electrodes are constructed mainly by two methods. First one is the modification of electrode by casting CNT dispersed solution on a solid substrate. One drawback of CNTs is its insolubility in most of the solvents. Hence ultrasonication is required to effectively disperse the tubes. This disadvantage can be surmounted by the functionalization of CNT or by the use of appropriate surfactants. To strengthen the mechanical connection between CNT and the electrode Nafion- a perfluorinate sulfonic acid ionomer is commonly used as a binder. The second method is to grow CNT electrode directly on a solid substrate. This ensures the absence of impurities originating from the surfactant and also provides good electrical contact between the sensing material and the electrode. The commonly used substrates used for modification with CNT are screen printed electrodes (SPE), glassy carbon electrodes (GCE), graphite electrodes and gold electrodes (Vashist et al., 2011). Recent researches are concentrating on the combination of CNTs and other smart nanomaterials to provide synergistic effects to enhance the sensor response.

2.1.1. Pristine carbon nanotube

CNT was used for the electrode modification in different types of sensors. Wang et al.used GCE modified with MWCNT for BRCA1 gene sensor with a signal enhancement in guanine oxidation signal (Wang et al., 2003). The detection was based on the guanine oxidation signal and the lower detection limit achieved was 100 fM. The electrochemical performance of unmodified and SWCNT/MWCNT modified screen printed electrode (SPE)was explored for DNA hybridization detection by measuring the magnitude of the oxidation signal of guanine and adenine (Erdem et al., 2012). It was observed that the electrode modified with MWCNT provide higher sensitivity and good reproducibility owing to the higher surface area provided by the MWCNT which leads to higher surface coverage of DNA.

All these experiments shed light on the use of CNT more specifically MWCNT as a suitable immobilization platform for DNA. Major challenge is to incorporate the biologically active molecules on the surface of nanotubes without altering the biological activity of the molecules while maintaining the electronic performance of the active layer. Various methods of modification of CNTs have shown to improve the sensitivity of the DNA sensor. In view of this the following section describe the various modifications of CNT used in DNA sensors and demonstrated that the modification of CNT lead to enhance the sensitivity of the sensor.

2.1.2. Functionalized carbon nanotubes

Functionalization of CNT provides more sites for covalent binding of biomolecules in addition to improving its biocompatibility (Balasubramanian and Burghard, 2005). Researchers have explored various kinds of functionalization methods of CNT and also different conjugation methods to immobilize DNA molecules on the CNT surface.

Carboxyl group functionalized CNTs have been used as the immobilization platform in DNA sensing. The carboxyl functionalized CNT provided an active surface area matrix for the binding of the ssDNA. SPE modified with carboxylic acid functionalized SWCNT was used for electrochemical detection of DNA hybridization related to the BRCA1 gene (Li et al., 2012a). Oxidized SWCNT immobilized on gold electrode through a self-assembled monolayer of cystamine for DNA hybridization detection (Zhang et al., 2011) also has been reported. In this sensor, quinine derivative is used as redox probe which is grafted onto the free carboxylic acid groups of the SWCNTs. After the hybridization with a complementary sequence, the redox kinetics of quinine was changed by the conformational changes of DNA and this leads to an increased current of the redox signal. A comparison between chemically functionalized and electrochemically functionalized MWCNTs for impedimetric DNA detection is done (Benvidi et al., 2015a). Here the MWCNT is functionalized with carboxyl group in both methods and compared the sensitivity for the DNA detection. The results showed that this DNA biosensor achieved a very promising detection limit of 0.124 attomolar (aM) with electrochemically functionalized MWCNTs and 1.33 aM with chemically functionalized MWCNTs.

Carboxyl group functionalized CNTs in combination with other molecules or nanomaterials also have been used. MWCNTs with carboxyl groups together with p-aminobenzoic and AuNPs was used to modify GCE for the detection of target DNA (Zhang et al., 2010). Aminobenzoic acid was electropolymerized on the surface of the functionalized MWCNTs which was immobilized on the GCE and then the surface is modified with AuNPs. Probe DNA was immobilized on