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Investigating the potential of multiwalled carbon nanotubes based zinc nanocomposite as a recognition interface towards plant pathogen detection

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ABSTRACT

The emergence of nanotechnology has opened new horizons for constructing efficient recognition interfaces. This is the first report where the potential of a multiwalled carbon nanotube based zinc nanocomposite (MWCNTs-Zn NPs) investigated for the detection of an agricultural pathogen i.e. Chili leaf curl betasatellite (ChLCB). Atomic force microscope analyses revealed the presence of multiwalled carbon nanotubes (MWCNTs) having a diameter of 50–100 nm with zinc nanoparticles (Zn-NPs) of 25–500 nm. In this system, these bunches of Zn-NPs anchored along the whole lengths of MWCNTs were used for the immobilization of probe DNA strands. The electrochemical performance of DNA biosensor was assessed in the absence and presence of the complementary DNA during cyclic and differential pulse voltammetry scans. Target binding events occurring on the interface surface patterned with single-stranded DNA was quantitatively translated into electrochemical signals due to hybridization process. In the presence of complementary target DNA, as the result of duplex formation, there was a decrease in the peak current from 1.89×10^{-04} to 5.84×10^{-05} A. The specificity of this electrochemical DNA biosensor was found to be three times as compared to non-complementary DNA. This material structuring technique can be extended to design interfaces for the recognition of the other plant viruses and biomolecules.

1. Introduction

Nanotechnology has emerged as one of the most exciting tools for the creation of efficient materials, devices, and systems through control of size at the 1–100 nm scale. Now a plethora of nanomaterials with different sizes, shapes, and compositions for a range of application are available (Khademhosseini et al., 2016). The exceptional properties of nanomaterials offer remarkable prospects for designing novel sensing systems, especially for improving the bioanalytical methods. Further, their properties can be tailored with respect to their size and structure which make them ideal candidates for devising innovative products.

During recent years, rapid and highly selective detection of DNA has gained much importance in the field of diagnostics, tissue matching, gene analysis, and forensic (Watson and Crick, 1953). On the basis of unique identity between the nucleic acid sequences of different organisms, diagnosis of several diseases is possible (Kogan et al., 1987). For the detection of nucleic acid based sequences, many techniques have been employed, however, polymerase chain reaction (PCR), gel electrophoresis, and membrane blot is among the most widely used techniques (Angelis et al., 1999; Beaudet and Belmont, 2008; Ding et al., 2009; Mullis and Faloona, 1987; Roberts et al., 2009; Southern, 1975). These conventional methods are accurate, but for routine analysis, these are slow and laborious, especially for the timely and rapid detection. To achieve this goal, nanotechnology based interfaces could offer favorable methods for DNA detection. Bio-affinity between nanomaterials and DNA molecules can be manipulated to design innovative biosensor platforms (Anzai, 2016; Bajwa et al., 2014; Dong et al., 2015; Juan-Colás et al., 2016). For the detection of DNA sequences, different biosensors e.g. plasma resonance, lateral flow, fluorophore-quencher, G-quadruplex, fluorescence resonance energy transfer (FRET) based biosensors have been reported (Chakraborty et al., 2015; Jensen et al., 2013; Jia et al., 2016; Jiang et al., 2014; Qiu et al., 2015). Among them, electrochemical biosensors are popular choices due to their sensitivity and rapidness (Gong et al., 2017; Shen et al., 2016; Wang et al., 2016). These sensors offer a cost-efficient, but a sensitive platform for the detection of various diseases like α and β-

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thalassemia (Chen et al., 2016). A glassy carbon electrode (GCE) modified with poly(2,6-pyridinedicarboxylic acid) (PDCA) can detect gemcitabine (GEM) up to 0.276 mg L^{-1} (Tığ et al., 2016). Whereas, a pencil graphite electrode (PGE) fabricated with DNA and Au nanoparticles has been developed in order to determine contamination caused by bacteria especially *Bacillus cereus* in dairy products (Izadi et al., 2016). An electrochemical biosensor based on a gold nanotube array (AuNTsA) has been used for the DNA detection of *Mycobacterium tuberculosis* with a detection limit of 0.05 ng μL^{-1} (Torati et al., 2016). For the assessment of total antioxidant capacity (TAC), a biosensor modified with dA_{20} (adenine-rich oligonucleotide) was used in commercial samples of juices (Cruz et al., 2016). In an effort to develop electrochemical DNA biosensor, graphene oxide/gold nanoparticles were used for the detection of *Helicobacter pylori* with a detection limit of 27 pM (Hajihosseini et al., 2016). Another innovative use of electrochemical DNA biosensor was to determine epidermal growth factor receptor *(EGFR)* exon 19 mutation (Xu et al., 2016). A biosensor is reported for the screening of DNA level modification and damage caused by chemotherapeutic drugs and a screen printed gold electrode (SPGE) for the detection of *Cucumber-Mosaic virus* (CMV) (Ilkhani et al., 2016; Zulkifli et al., 2016). Despite the material advancements very scanty data is available where electrochemical biosensors have been applied for the detection of agricultural pathogens for their rapid and timely identification to prevent huge economic losses.

Among nanomaterials, multiwalled carbon nanotubes (MWCNTs) are particularly attractive for bioelectronic detection. Their electronic conductance is strongly responsive to any small change in the surface chemistry e.g. attachment of any ligand or biomolecule to their surface. Various novel systems can be designed by coupling high surface-to-volume ratio, outstanding electron transport properties, ease of manipulating the rich chemistry of MWCNTs with other nanostructures, and biomolecules. The resultant tailored electronic conductivity of MWCNTs makes them extremely attractive material for constructing better electrochemical sensing devices. Purposely designed and loaded nanoparticles on MWCNTs can serve as an amplification source and thus can lead to sensitive detection of bioanalytes like DNA.

Timely identification of a pathogen plays a very important role in an agricultural production system. *Chili leaf curl virus* is one of the important viruses belongs to genera *Begomovirus* of the family *Geminiviridae* that causes chili leaf curl disease in chili pepper (*Capsicum annum* L.) (Briddon et al., 2003; Shih et al., 2003). The begomoviruses are either bipartite or monopartite. In bipartite begomovirus, there are two main genomic contents i.e. DNA-A and DNA-B. However, monopartite begomoviruses are those begomoviruses that comprises of only single genomic content which has homogeneity to bipartite *Begomovirus* DNA-A component (Stanley et al., 2005). The *Begomovirus* complex comprises of alphasatellites and betasatellites which are small satellite like molecules involve in self-replication and to determine the pathogenicity respectively (Jyothsna et al., 2013; Mansoor et al., 1999). In many areas of the world, Chili leaf curl betasatellite (ChLCB) is the dominant one and it causes infection in chili (Hussain et al., 2009).

Here in this study, we have introduced the designing of a microenvironment based on novel MWCNTs, zinc nanoparticles (Zn-NPs), and methylene blue (MB). This is used as a recognition interface for a DNA sequence with improved response characteristics. The electrochemical DNA biosensor developed thus represents a simple, straightforward approach for the detection of the sequence of interest. This protocol has the potential for establishing as a diagnostic tool for other biomolecules as well.

2. Experimental

2.1. Reagents and chemicals

MWCNTs, phosphate buffered saline (PBS) pH = 7.4, 0.1 M methanol solution of zinc chloride ($ZnCl_2$), polyethylene imine (PEI), 0.1 M sodium borohydride ($NaBH_4$) solution, sodium dodecyle sulfate (SDS), 1 M potassium nitrate (KNO_3), 50 mM PBS solution of potassium ferricyanide ($K_3Fe(CN)_6$), 5 mM methylene blue (MB), 1 M nitric acid (HNO_3), silver chloride (AgCl), PCR green mixture and short oligonucleotides were purchased from Shenzhen Nanotech. Port. Co. Ltd. (China), Sigma-Aldrich, Eurofins MWG Operon, and Merck. The ultra pure water obtained from Barnstead™ Smart2Pure™ was used throughout the experiments (Thermo Scientific).

The primers used for the amplification of probe and complementary target DNA from the clone of ChLCB (Acc. No. FR751147). The set of primer sequences used in this study for the amplification of ChLCB is CbC1F (5′TAT-GAATCGATATGCACCACGTATATGAA 3′) CbC1R (5′ATACTG-TCGACTCACACACACACATTCGTAC 3′) (Tahir et al., 2011).

Similarly, for the amplification of cotton leaf curl Multan betasatellite (non-complementary DNA) from clone LN870411, following set of primers are used, i.e. β 01 (5′ GGTACC ACTACGCTACGCAGCAGCC 3′) and β 02 (5′ GGTACC TACCCTCCCAGGGGTACAC 3′) (Briddon et al., 2002).

2.2. Instrumentation

Electrochemical investigations were carried using Autolab Potentiostat/Galvanostat (PGSTAT) with in-built the General Purpose Electrochemical System software (GPES version 4.9). For data treatment, a linear baseline correction was applied in the case of cyclic voltammetry and the data were smoothed by the Savitzky and Golay method (level 04).

For the amplification and quantification of DNA sequences, Thermal cycler C1000 Touch™ (BIO-RAD, USA) and NanoDrop™ 1000 spectrophotometer (Thermo Scientific) were used, respectively. In order to characterize the nanocomposite, different techniques have been applied. The surface charge on the as-synthesized composite was determined by Zetasizer Nano ZS (Malvern) which works on the principle of dynamic light scattering. The morphology was studied with field emission scanning electron microscope (FE-SEM-JSM-7500F-JEOL, Japan) and atomic force microscope (AFM- SHIMADZU WET-SPM 9600, Kyoto, Japan).

2.3. Preparation of MWCNTs-Zn NPs composite

To create reaction centers for the loading of nanoparticles, amine functionalization of MWCNTs was carried out by following a protocol (Hameed et al., 2017). Briefly, 0.05 g of MWCNTs was dissolved in 5 mL PEI solution (1% v/v) prepared in methanol, followed by the incubation for 2 h at room temperature. Afterwards, this mixture was further dissolved in 40 mL of methanol till stable colloidal solution is formed. Then $ZnCl_2$ (10 mL; 0.1 M) was added into it, followed by the drop-wise addition of $NaBH_4$ solution (30 mL; 0.1 M) in the mixture. Finally, MWCNTs were washed with methanol and water (15 mL each), and then put in a vacuum concentrator for drying at 50 °C.

2.4. Fabrication of electrochemical DNA biosensor

For the preparation of electrochemical DNA biosensor, the surface of GCEs was polished by using 0.05, 0.3 and 1 μm alumina slurries. After that GCEs were cleaned by sonication for 5 min in ethanol, HNO_3 (1 M) and water (5 mL each, in batch mode). For the characterization of GCEs cyclic voltammetry were recorded between −1 to 1 V in $K_3Fe(CN)_6$ (50 mM) and KNO_3 (1 M) solution against AgCl (reference electrode) at a scan rate of 100 mVs^{-1} scan rate.

The amplification of ChLCB was done using the specific set of primers CbC1F/CbC1R by following PCR profile, 94 °C for 5 min (preheat treatment) followed by 34 cycles of 94 °C for 30 s (denaturation), 52 °C for 30 s (annealing), 72 °C for 45 s (extension) and final extension at 72 °C for 10 min (Tahir et al., 2011). Amplified product (1.4 kb) was