Journal of Hazardous Materials 346 (2018) 27–35

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Evaluation of carbon nanotube based copper nanoparticle composite for the efficient detection of agroviruses

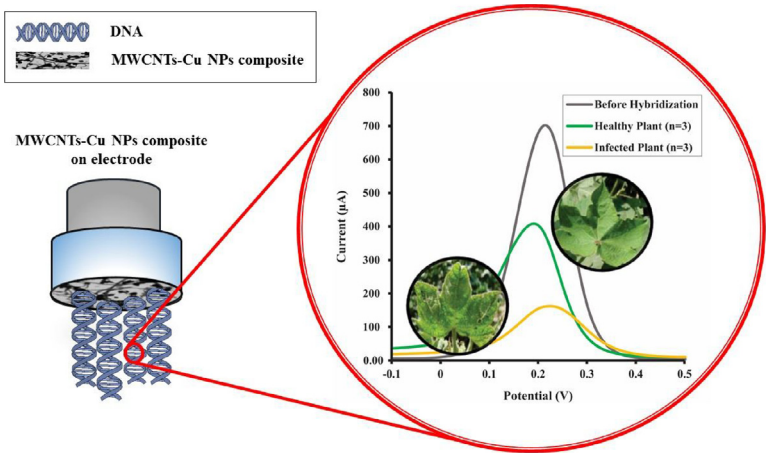
Muhammad Ali Tahir[a,c,d], Sadia Z. Bajwa[a], Shahid Mansoor[a], Rob W. Briddon[a], Waheed S. Khan[a], Brian E. Scheffler[b], Imran Amin[a,*]

[a] National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan
[b] USDA ARS, Genomics and Bioinformatics Research Unit, P.O. Box No. 36, Stoneville, MS 38776, USA
[c] Pakistan Institute of Engineering and Applied Sciences (PIEAS), Nilore, Islamabad, Pakistan
[d] Department of Environmental Science and Engineering, Fudan University, Shanghai, 200433, P.R. China

HIGHLIGHTS

- Carbon nanotubes based copper nanoparticles composite can be used to develop biosensor for the detection of a begomovirus (CLCuKoV-Bur).
- The morphology of composite consists of copper nanoparticles (20–100 nm) anchored along the whole lengths of tubes.
- The developed sensor exhibits excellent ability to detect CLCuKoV-Bur selectively up to 0.01 ng μL^{-1} DNA concentration.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 July 2017
Received in revised form
30 November 2017
Accepted 3 December 2017
Available online 5 December 2017

Keywords:
Carbon nanotube composite
Copper nanoparticles
Electrochemical detection
DNA biosensor
Agroviruses

ABSTRACT

We report a biosensor that combines the construction of a three-dimensional nanocomposite with electrochemical methods for the detection of viruses in plants. This is the first report, where carbon nanotubes are used as a conductive frame to anchor highly electrolytic agglomerates of copper nanoparticles to detect agroviruses. Morphological analysis of nanocomposite revealed the presence of carbon nanotubes having a diameter of 50–100 nm with copper nanoparticles of 20–100 nm, attached in the form of bunches. This material was applied to assess the infection caused by geminiviruses which are a major threat to the cotton plants in Asian and African countries. The hybridization events were studied by monitoring differential pulse voltammetry signals using methylene blue as a redox indicator. In the presence of target DNA, sensor signals decreased from 7×10^{-4} to 1×10^{-4} Ampere. The probe exhibited 97.14% selectivity and the detection limit was found to be 0.01 ng μL^{-1}. The developed biosensor is stable for at least four weeks, losing only 4.3% of the initial signal value. This sensor was able to detect the presence of viruses in sap extracted from cotton leaves, thus providing a promising platform to detect a range of other crops-infecting viruses.

* Corresponding author at: Department of Environmental Science and Engineering, Fudan University, Shanghai, 200433, P.R. China.
E-mail address: imranamin1@yahoo.com (I. Amin).

https://doi.org/10.1016/j.jhazmat.2017.12.007
0304-3894/

1. Introduction

The interaction between nanomaterials and bio surfaces can be harnessed for the development of efficient interfaces for optical, electrical, mechanical, and electrochemical detection of various biologically important molecules and cells [1]. In particular, cost-efficient, but selective, nano/bio interface based DNA sensors have attracted much attention as several diseases can be identified by the recognition of unique DNA sequences of a particular pathogen [2]. The basic phenomenon in most types of DNA detection technology is based on the hybridization between a specific DNA probe sequence and its target complementary strand. Various techniques have been developed for DNA identification and among them, the most commonly used technique is polymerase chain reaction (PCR), among other traditional methods like gel electrophoresis or membrane blots [3–5]. However, these methods are slow and laborious for routine analysis and especially for the timely and rapid detection of specific DNA targets. Therefore, there is a dire need to develop a detection method that is simple, low cost as well as accurate and sensitive. To achieve this goal, nanotechnology based interfaces could be one of the efficient options.

Advanced nano-biosensors are versatile tools to develop diagnostic systems for a variety of analytes [6–8]. For the detection of specific DNA sequence or mutated genes associated with infection or disease, DNA biosensors are popular choices because base pairing interactions between complementary sequences are highly specific and faster. The performance and sensitivity of electrochemical biosensor can be improved further by incorporation of functionally important nanomaterials to develop interfaces *e.g* graphene oxide based biosensor based on linkage of graphene oxide sheets with iridium single-stranded DNA (ssDNA) for the detection of target ssDNA [9]. An electrochemical biosensor based on DNA polymerase and HRP-SiO_2 nanoparticles (NPs) has been fabricated for the detection of point mutation in the K-ras gene [10]. Among nanomaterials, carbon nanotubes (CNTs) have attracted greater attention of the scientific community. Their small dimensions, strength and the remarkable physical and chemical properties make them a model candidate for a whole range of promising applications [11]. Here, the increased surface area of CNTs can additionally be used for immobilization of large amounts of a bio probe especially for detection purpose [12]. CNTs cannot be directly employed for DNA detection and other biological reactions because the surfaces of CNTs are chemically inert. However, these can be modified or coated with functional materials for efficient detection [13].

Timely identification of a pathogen can be critical in controlling a disease outbreak in an agricultural production system [14]. Cotton is an important renewable natural fiber, but its production is adversely affected by cotton leaf curl disease (CLCuD) complex, which is basically the group of viruses that belongs to genus *Begomovirus* of the family *Geminiviridae* [15,16]. This disease is spreading fast and has become a threat to the production of cotton in several countries of Asia, South East Asia, and Africa [17].

A large variety of metal nanoparticle-based techniques has been developed for DNA detection since last decade. These techniques received popularity as nanomaterials offer miniaturization and cost-efficient detection along with high sensitivity and specificity. Their unusual optical and electrical properties, their small label size, bioconjugation chemistry make them an exceptional tool for DNA detection [18,19]. These astounding properties of nanomaterials can be assimilated with the electrochemical transducer to construct efficient and sensitive DNA biosensors.

To the best of our knowledge, the present study is the first report describing a functional interface of multi-walled carbon nanotubes copper nanoparticles (MWCNTs-Cu NPs) composite to assess the infection caused by an agriculture related pathogen. For enhanced performance of interfaces, electrical features of metal nanoparticles are combined with high conductivity of MWCNTs. These nanostructures are used to electrostatically bind a probe, specific to virus strain, and the complementary virus DNA. This interface served as a recognition layer of malicious DNA, while greatly amplifying the DNA sensor response due to the synergistic effects of MWCNTs and Cu NPs. To exploit DNA assisted charge transport chemistry upon exposure to complementary DNA, hybridization sequences are monitored as sensor responses by recording electrochemical signals. Further, in the same study, we demonstrate how this DNA-mediated charge transport at the nano-bio interface can be implemented to produce a sensitive assay for the detection of infection in cotton plants.

2. Experimental

2.1. Reagents and materials

All chemicals were purchased either from Merck, Sigma-Aldrich or mentioned otherwise. All the solutions were prepared with ultrapure water from Barnstead™ Smart2Pure™ (Thermo Scientific).

The oligonucleotide sequences used in this study are as follows:

Probe DNA: *Cotton leaf curl Khokran virus*-Burewala strain (CLCuKoV-Bur) clone (acc. no. AM774294)

Complementary Target DNA: CLCuKoV-Bur clone (acc. no. AM774294)

Non-complementary DNA: GroEl gene (acc. no. AF130421). Further specific details of amplification can be found in the supplementary information (SI; sections 1.1 and 1.2).

2.2. Instruments

In this work, Potentiostat/Galvanostat Autolab PGSTAT-12 (Metrohm Autolab B. V.), glass vessel (6.1415.210 Metrohm USA Inc.), Zetasizer Nano ZS (Malvern), thermal cycler C1000 Touch™ (BIO-RAD, USA), NanoDrop™ 1000 spectrophotometer (Thermo Scientific, Wilmington, USA) and Fourier transform infrared spectrometer (FT-IR) model ALPHA FT-IR (Bruker Corporation) were employed. For morphological characterization of MWCNTs-Cu NPs composite and MWCNTs a field emission scanning electron microscope (FESEM) model JSM-7500F (JEOL, Japan) equipped with a transmission electron detector was used. Root mean square (RMS) was calculated using an atomic force microscope (AFM) model SHIMADZU WET-SPM 9600 (Kyoto, Japan).

2.3. Preparation of MWCNTs-Cu NPs composite

First, MWCNTs were functionalized and to do this, MWCNTs (0.03 gm) were dispersed in 5 mL methanol solution of PEI (1% v/v). The mixture was sonicated for 2 h and then maintained at room temperature for another 2 h. Next, these MWCNTs wrapped with PEI, were dispersed in 40 mL methanol until a stable colloid was formed. After colloid formation, $CuCl_2$ solution (0.1 M in methanol; 10 mL) was added to the mixture and stirred for 30 min at room temperature. Then $NaBH_4$ solution (0.1 M in methanol; 30 mL) was added drop-wise, followed by stirring for 10 min. Finally, the suspension was washed with 15 mL each of methanol and H_2O, and then dried in a vacuum concentrator at 50 °C.

2.4. Preparation of DNA probe

Glassy carbon electrodes (GCEs) were treated consecutively with 1.0, 0.3 and 0.05 μm of alumina slurries for 1 min each. The electrodes were washed with water, ethanol, and 1 M HNO_3 before being dried in a nitrogen atmosphere. After cleaning, the surfaces of the GCEs were characterized by recording cyclic voltammetry (CV)