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Chemiresistive biosensors based on carbon nanotubes for label-free detection of DNA sequences derived from avian influenza virus H5N1

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ABSTRACT

We developed chemiresistor-type biosensors based on carbon nanotubes for highly efficient and fast detection of avian influenza virus (AIV) subtype H5N1 DNA sequences. Semiconducting single-walled carbon nanotubes (sc-SWCNTs) or nitrogen-doped multi-walled carbon nanotubes (N-MWCNTs) were used as two alternative active sensing elements, and their sensitivity to different concentrations of DNA target were compared. In these sensors long nanotubes (>5 μm) were placed between interdigitated metal electrodes so that individual nanotubes connect the electrodes. The nanotubes were functionalized with DNA probe sequences non-covalently attached to the sidewalls. Such functionalized-nanotube sensors could reliably detect complementary DNA target sequences of the AIV H5N1 with concentration ranging from 2 pM to 2 nM in 15 min and at room temperature. Our nanotube-based biosensors are small, flexible, disposable and easy-to-fabricate that makes them promising for point-of-care applications and clinical diagnostics.

1. Introduction

Avian influenza virus (AIV), especially subtype H5N1 has become nowadays a problem not only for poultry because of its pathogenicity and relatively high lethality rate among human. Highly pathogenic AIV H5N1 can infect people and can be transmitted from human to human with disastrous implications for public health [1,2]. High-sensitive and rapid detection of H5N1 infection would allow early antiviral therapy and control of the outbreaks [3–6]. However, the most commonly used detection techniques are laborious, time-consuming, or require specialized laboratory facilities and well-trained technical personnel, which greatly limits their application in clinical tests [3–5]. Direct DNA hybridization biosensing has become recently a very promising approach, not only because of its high sensitivity, but also of label-free detection and easy integration with other, in particular portable devices, which makes it a promising candidate for the development of an integrated, high-throughput, low-cost and tiny AIV biosensor for "point-of-care" diagnostics [7–10].

Nowadays, DNA hybridization chemiresistive nanosensors have been developed, which are very attractive because of their reduced size, high sensitivity and a simple detection principle based on the change of resistance in response to the binding of DNA target (DNA T) to active nanomaterials [10–13]. Recently, there has been a rise in the use of one-dimensional nanostructures such as silicon nanowires (Si-NW) [8,14], conducting polymer nanowires [11,15] and carbon nanotubes (CNTs) [16–19] as transducing elements in chemiresistive sensors. Among these materials, CNTs, especially single-walled CNTs (SWCNTs), have increasingly garnered considerable interest from the DNA sensor research due to their high aspect ratio, good environmental stability, excellent mechanical and electronic properties as well as the convenience for label-free sensing, which hold a great potential for integrating them into com-

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pact, low-power and portable miniaturized sensors [13,17–20]. However, relatively strong van der Waals interaction between CNTs leads to agglomeration of CNTs in both powder and dispersion form thank to their small size and high aspect ratio. This makes individualization, processing and integration of CNTs quite challenging that becomes finally a bottleneck in a scalable fabrication of CNT-based sensing devices [21,22].

Herein we report on the development of stable chemiresistor-type sensors based on semiconducting SWCNTs (sc-SWCNTs) and nitrogen-doped multi-walled CNTs (N-MWCNTs) for highly sensitive and rapid label-free detection of AIV H5N1 virus. Our sensor fabrication technology overcomes the difficulty of integration of CNTs into devices; relatively long nanotubes with lengths exceeding 5 μm directly connect metallic electrodes. Since DNA are charged molecules, field effect is expected to play the major role in influencing electronic structure of nanotubes. Therefore, sc-SWCNTs were applied in this work as they showed a higher sensitivity than pristine SWCNTs when gas and bio-molecules are adsorbed to their surface [23–25]. On the other hand, N-MWCNTs were employed as they have amine groups on their sidewalls which would facilitate functionalization of nanotubes with DNA probe [8]. However, doping with nitrogen modifies electronic performance of nanotubes compared to undoped ones [26–28] which may lead to a decrease in sensitivity. In case of N-MWCNT, well-aligned nanotube arrays placed between interdigitated electrodes were fabricated on both rigid and flexible substrates that was achieved by contact-printing of vertically-grown N-MWCNTs onto a clean target substrate. The sensors with sc-SWCNTs were fabricated on quartz substrates only as these substrates served as a support for sc-SWCNT growth.

2. Experimental

2.1. Materials

All the reagents were of analytical grade and were used without further purification. Three series of the samples were fabricated: on glass and quartz substrates of about 1 mm thick (QSIL GmbH, Germany) as well as on 100 μm thick polyimide foils Kapton® (Dupont HN100). The latter were applied for the fabrication of flexible device. Acetonitrile and ethanol (Sigma–Aldrich) were used for synthesis of N-MWCNTs. ProNT™ sc-SWCNTs on quartz substrates with a length from 5 to 20 μm were produced by ProNT GmbH. No surfactants or catalyst particles were present on ProNT™ sc-SWCNTs. Single-stranded DNA of AIV H5N1 sequences were purchased from Eurofins Genomics, with the base pair sequences: 5′-CAA ATC TGC ATT GGT TAT CA-3′ for DNA probe, and 5′-TGA TAA CCA ATG CAG ATT TG-3′ for DNA T. For negative control AIV H1N1 DNA T sequence 5′-GTA GGT TGA CAG AGT GTG-3′ was used which was non-complementary to the H5N1 DNA probe. The probe and DNA T were diluted with phosphate buffer (PB) solution to a final concentration of 20 μM for further use. Triton™ (4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol, Sigma-Aldrich) 0.01% in buffer was used to block the uncovered sites at nanotube sidewalls.

2.2. N-MWCNT growth, characterization and contact printing

Vertically aligned N-MWCNTs were synthesized by means of chemical vapor deposition (CVD) on a Si/SiO_2 wafer surface with pre-deposited Fe/Al_2O_3 metallic-layer serving as the source of catalyst. 1 nm thick Fe layer was deposited after 10 nm thick Al_2O_3 layer was built on the 400 nm thick SiO_2 surface of the wafer by magnetron sputter deposition in high vacuum chamber (base pressure: 10^{-7} mbar; Ar sputter pressure: 10^{-3} mbar). Acetonitrile/ethanol mixtures were employed as C/N source, the doping content of N-MWCNT structure can be controlled through varying the proportion of acetonitrile to ethanol [28–30]. The CVD was performed between 760 and 910 °C (synthesis temperature was closely related to acetonitrile/ethanol ratio in a precursor solution and was optimized for each acetonitrile/ethanol ratio) for 15 min with a pressure of 100 mbar. After the growth, the morphology and density of vertical nanotube "forest" was analyzed with scanning electron microscope (SEM), see Fig. 1(a). X-ray photoelectron spectroscopy (XPS) measurements were performed to determine the chemical constitution of N-MWCNT samples (a PHI 5600 CI system with a hemispherical energy analyzer was used). According to XPS investigation, the N doping concentration was varied from 6.14% to 9.34% when the fraction of acetonitrile in ethanol in the precursor was varied from 20% to 50% (see Table 1). Samples of vertically grown N-MWCNTs with different content of nitrogen have been synthesized.

Table 1
N doping content in N-MWCNTs produced from varying N-containing feedstocks.

Nr.	Acetonitrile percentage in ethanol (vol%)	N doping content (at%)
1	50	9.34
2	30	8.20
3	20	6.14

In order to form horizontally aligned N-MWCNT arrays, direct contact printing was applied for transferring as-grown vertically aligned nanotubes onto various clean target substrates similar to contact-printing of Si-NW reported elsewhere [8]. The "ink" for the direct contact printing is a dense "forest" of vertically aligned N-MWCNT arrays with the height of 5–10 μm. Target substrate applied here were glass, quartz and Kapton® polyimide foil, which were first cut into pieces with a size of 20 × 20 mm and then cleaned with ultrasonic washer in an acetone, ethanol and deionized water bath successively for 10 min. Octadecyltrichlorosilane (C18H37SiCl3) (ODTS) was applied to chemically modify the surface of rigid target substrates and to increase the adhesion of N-MWCNTs. The main steps of direct contact printing are schematically illustrated in Fig. 1(b). During the contact printing process, an intimate contact at the interface between the growth and target substrates causes a relatively strong van der Waals interaction between nanotubes and target substrate that leads to the detachment of nanotubes from the growth substrate and binding them to the surface of the target substrate. Besides adhesion strength, the surface density of horizontal N-MWCNT arrays was controlled by adjusting CVD process parameters to synthesize vertically aligned N-MWCNTs with optimal length and surface density as well as external pressure applied to the substrate with vertically aligned N-MWCNTs during contact printing onto the target substrate. The results of direct contact printing for external pressure ranging from 0.5 to 3.0 kPa can be seen in Figure S1 of Supplementary data. For the fabrication of our N-MWCNT-based sensors external pressure of 1 kPa was utilized.

In case of sc-SWCNT, no horizontal alignment was achieved. In these samples sc-SWCNTs of 5 to 20 μm in length were obtained by CVD-based epitaxial elongation of sc-SWCNT short fragments (seeds) chaotically distributed on a quartz substrate and were directly used for device fabrication. It is to emphasize, that no surfactants were present on sidewalls of nanotubes in all samples (M-NWCNTs and sc-SWCNTs) before functionalization step (described below).

2.3. Sensor fabrication and device layout

The chemiresistor-type sensors were fabricated using a standard microfabrication procedure. After forming CNTs arrays on the target substrates, Cr/Au (3 nm Cr and 50 nm Au) interdigitated