The Effect of Network Density on the DNA-Sensing Performance of Single-Walled Carbon Nanotubes

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Received: August 20, 2009; Revised Manuscript Received: October 15, 2009

The effect of network density of single-walled carbon nanotubes (SWNTs) on the detection of DNA hybridization was investigated. The results show that, in contrast to those having higher densities, SWNTs with low network densities in the conductance range of $0.74 \times 10^{-7} < G_{\text{bare}} < 2.00 \times 10^{-7}$ exhibit high sensitivity for detection of immobilized DNAs. The resulting SWNT devices with optimal network densities showed good selectivity in detecting cDNA hybridization. The network density control will provide opportunities to realize practical label free biosensor utilizing commercially available SWNT networks.

I. Introduction

Single-walled carbon nanotubes (SWNTs) have attracted considerable attention as a platform material for manufacturing miniaturized label-free biosensors. Their flexible surface chemistry, large surface areas, and excellent electronic properties allow the detection of various biomolecules in a wide range of formats, including electronic sensing devices. DNA and proteins have been successfully immobilized on SWNT surfaces by various noncovalent and covalent binding methods. Noncovalent adsorption through π stacking or hydrophobic interactions has been employed as a nondestructive functionalization method in the construction of field effect transistor (FET) based biosensor. On the other hand, covalent bonding method was used to provide more stable and site-specific linkages between biomolecules and SWNTs.

For practical applications of SWNTs in manufacturing electrical biosensor, formation of SWNT networks is essential. In contrast to an individual nanotube, which has a high risk of channel failure and requires a labor-intensive process for device fabrication, nanotube networks are suitable for reproducible, cost-effective mass production. Also, large assemblies of nanotubes can be tailored to have desirable sizes and have abundant surface reaction sites to which sufficient amounts of biomolecules can be linked.

Introduction of biomolecules to the surface of nanotube network is normally accompanied by considerable changes in electrical signals, a phenomenon that is attributed to changes in the electrical transport properties of the nanotube caused by linked molecules. It is also known that the electrical behavior of the nanotube films is strongly dependent on network density. The SWNT networks display metallic behavior and thus show linear current−voltage (I−V) responses in specific density regions. On the other hand, nonlinear I−V behavior is seen in networks having densities close to the percolation threshold. As a consequence of these characteristics, the SWNT network density might influence the sensitivity of electrical detection of biomolecules immobilized on SWNT films. Despite its significance to the design of practical label-free biosensors, however, it has not been understood how network density governs the biological sensing performance of SWNTs.

In this study, we investigated the effect of SWNT network density on the DNA-sensing performance. By systematically modulating the SWNT film density, we found that the SWNTs with network densities in a low conductance range were superior for detection of DNA that is complementary to immobilized DNA.

II. Experimental Methods

Materials. The SWNTs containing raw soot (85 wt % purity, prepared by a high-pressure carbon monoxide process) were purchased from Carbon Nanotechnologies, Inc., USA. N,N-Dimethylformamide (DMF) (99.5%), sodium hydroxide (NaOH, 97%), hydrogen peroxide (H$_2$O$_2$, 35%), sulfuric acid (H$_2$SO$_4$, 95%), and hydrochloric acid (HCl, 35%) were obtained from Junsei Chemical Co., Ltd. Aminopropylsilane-modified glass slides (Corning GAPS II coated slides, 25 × 75 mm) were obtained from Corning Incorporated. DNA oligonucleotides were purchased from GenoTech Co. (Daejeon, Korea). The 3’ termini of the probes DNA molecules (Table 1) were modified with amine residues for their immobilization on the SWNT films by forming amide linkages. 4-Morpholineethanesulfonic acid (MES), N-hydroxysuccinimide (NHS), and 1-(3-dimethylamino-nopropyl)-3-ethycarbodiimide hydrochloride (EDC) were purchased from Sigma.

Preparation of SWNT Film. The SWNTs were purified by dry oxidation at 365 °C for 90 min in a flow of dry air (0.1
TABLE 1: Synthetic DNA Oligonucleotides Used in the Fluorescence and Electrical Detection Experiments

<table>
<thead>
<tr>
<th>name</th>
<th>sequence (5’→3’)</th>
</tr>
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<tbody>
<tr>
<td>P1</td>
<td>Cy5-TGTGCGACCATCAACCTGTG-amine</td>
</tr>
<tr>
<td></td>
<td>TGTGCGACCATCAACCTGTG-amine</td>
</tr>
<tr>
<td>P2</td>
<td>Cy5-CACAGGTTGATGGTCGCCACA-amine</td>
</tr>
<tr>
<td></td>
<td>CACAGGTTGATGGTCGCCACA-amine</td>
</tr>
<tr>
<td>target</td>
<td>Cy5-CACAGGTTGATGGTCGCCACA</td>
</tr>
<tr>
<td></td>
<td>CACAGGTTGATGGTCGCCACA</td>
</tr>
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carboxylic acid groups, the SWNT films were treated with MES buffer solution containing NHS and EDC. After spotting 50 μM of probe DNA in MES buffer solution followed by incubation, the films were washed with MES buffer solution to remove unbound DNA. The second approach used for the immobilization of DNA relies on noncovalent attachment via adsorption onto nanotube surfaces through π−π interactions between aromatic bases of DNA and the sidewalls of the carbon nanotubes. For this purpose, probe DNAs in buffer solution (Tris, EDTA, NaCl) were spotted on the SWNT films and incubated on the SWNT films with the same concentration as that of covalent binding. Synthetic DNA probes (P1 and P2) labeled with Cy5 (Table 1) were employed for examining immobilization efficiency via fluorescence measurements.

The fluorescence images and corresponding fluorescence intensities obtained with the nanotube network films having covalently and noncovalently immobilized DNA are shown in Figure 3a. The fluorescence intensities were remarkably high when DNA was covalently linked. In contrast, noncovalent adsorption of DNA increased the fluorescence to a slightly lower level, indicating a lower efficiency of immobilization.

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Figure 1. Overall scheme for fabrication of SWNT film based, two-terminal devices for detection of DNA hybridization. (a) Fabrication of film consisting of short SWNT strands by using the vacuum filtration method. (b) Photoresist coating, mask alignment, and UV exposure process on the SWNT films. (c) Development process. (d) Removal of the unprotected nanotube films via RIE. (e) Mask alignment and UV exposure process, (f) Removal of exposed photoresist by developer. (g) Metal evaporation of Co/Au. (h) Lift-off process through dissolution of the photoresist. (i) Covalent binding between amine terminated DNA oligonucleotides and carboxylic acid groups of SWNTs (pink circles: carboxylic acid group; blue circles: amine group; purple diamonds: amide bonding). (j) Hybridization of target DNA molecules.

Figure 2. (a) An optical microscopy image of two-terminal resistors used for label-free DNA detection. (b) A scanning electron microscopy (SEM) image of the electronic DNA sensor consisting of linear patterned SWNT films bridging two electrodes. (c) A close-up SEM image of the junction between metal electrodes and an SWNT film patterned by RIE. (d) A higher magnification SEM image showing clear boundaries between the substrate and patterned networks piled by strands of shortened SWNT.

Figure 3. (a) Fluorescence intensities and scanned fluorescence image (inset) after immobilization of Cy5-labeled probe DNA onto SWNT film by using both the covalent and noncovalent binding method. The inset of (a) is a scanned fluorescence image resulting from respective binding methods. (b) Fluorescence intensities after hybridization of Cy5-labeled target DNA molecules onto SWNT film that was both covalently and noncovalently immobilized with the two kinds of unlabeled DNAs: one a probe DNA having complementary sequences (P1), the other a probe DNA containing a noncomplementary sequence (P2) to the target DNA. The inset of panel b is a scanned fluorescence image resulting from the spots of cDNA (C (P1)) and non-cDNA (NC (P2)).
higher level than the background. These findings indicate that
most of the covalently linked DNA molecules are strongly
bonded as compared to physisorbed DNAs, resulting in high
efficiency of immobilization. Raman and IR spectra results
further confirm the covalent bonding between DNA and SWNT
(Supporting Information, Figures S1b and S3). To further
examine the efficiencies of DNA sequence specific hybridization,
unlabeled DNAs that are complementary (P1) and non-
complementary (P2) to the target DNA were immobilized on
SWNT films by using the covalent and noncovalent binding
methods (Figure 3b). The DNAs immobilized on the SWNT
films were then hybridized with the Cy5-labeled target DNA.
After hybridization of the target DNA to the complementary
probe DNA covalently immobilized on the SWNT film, the
mean fluorescence intensity became about 30 times higher than
that observed for the noncomplementary probe DNA, indicating
the successful hybridization of target DNA to cDNA. In contrast,
the fluorescence intensity of the SWNT film with noncovalently
bound DNA displayed no distinctive difference when hybridized
with the complementary and noncomplementary probe DNAs.
It is likely that the noncovalent binding of DNA to SWNTs
leads to unstable adducts and, as a result, that DNA molecules
adsorbed on the SWNT surface were removed during the
hybridization and/or repeated washing steps. The results show
that the films with covalently linked DNA exhibit higher
selectivity for detecting hybridization with cDNA versus non-
cDNA. Thus, the covalent binding method is superior to the
noncovalent method for the immobilization of DNA molecules
onto the SWNT films.

The effect of the SWNT network density on the electrical
response of covalently immobilized DNA sensing devices is
shown in the images and plots given in Figure 4. For the purpose
of statistical analysis, 120 resistors having various densities of
nanotube networks were fabricated by employing SWNT
suspensions having different concentrations (Figure 4a). The
resulting devices are comprised of eight different network
densities. The electrical responses from each network density
are the averages of 15 different circuits. Before immobilizing
DNA molecules on the nanotube networks, the electrical
behavior of the SWNT films as a function of network density
was examined. Current–voltage (I–V) plots arising from the
SWNT networks were linear (Figure 4b), suggesting that the
devices exhibit conductance properties that result from the
nanotube networks containing one-third metallic and two-thirds
semiconducting SWNTs over the percolation threshold.19 As
the network density increased, the slope of the corresponding
I–V plots (electrical conductance \(G[S]\)) increased dramatically.

A significant finding is that the DNA sensing performance
of the SWNT-based device was strongly affected by the
nanotube network density, as demonstrated by the data included
in Figure 4c. To estimate the change in conductance response
promoted by the immobilization of DNA, we used the following
equation:

\[
\frac{|G_{\text{immo}} - G_{\text{bare}}|}{G_{\text{bare}}} \times 100(\%)
\]

where \(G_{\text{bare}}\) and \(G_{\text{immo}}\) represent the respective conductance
of the as-fabricated nanotube network and the DNA immobilized
film.

Note that each point in the plot given in Figure 4c represents
a mean percentage change in the conductance response of the
films with the given network density. The results show that the
countenance responses were low (5%) in films with high
network densities (conductance in the range of \(4.58 \times 10^{-7} < G_{\text{bare}} < 8.50 \times 10^{-7}\)). In the low network density region (0.74
\(\times 10^{-7} < G_{\text{bare}} < 2.00 \times 10^{-7}\)), however, the conductance
response dramatically increased as the network density increased.
The response reached a maximum (80%) at a network density corresponding to \(G_{\text{bare}} \sim 1.0 \times 10^{-7}\), after which it
decreased as the network density increased. In contrast, almost
no response was observed in the high network density region
\(G_{\text{bare}} \sim 4.50 \times 10^{-7}\).

This observation suggests that there is an optimum range of
SWNT network density for the effective detection of DNA
immobilization. The DNA sensing performance was much better
for devices with relatively low network densities as compared
to those with high network densities. This behavior is likely
due to the result of a balance between the number of reaction
sites and the conductance of the network film. When the network
density increases, both the conductance of the SWNT film and
the number of DNA molecules attached on the film increase.
However, the nanotube network is already highly conductive
when its density is high. As a result, immobilization of DNA
does not significantly alter the conductance even though a large
number of DNA molecules are present on the surface. In
contrast, a low-density nanotube network has a relatively low
indigenous conductance. Thus, the change in conductance after DNA immobilization becomes highly sensitive to the relative amount of DNA that is bound. Owing to this phenomenon, there is a specific range of SWNT network density that enables sensitive recognition of immobilized DNAs. On the basis of the experimental results, it appears that the conductance of the device needs to be in the range of $0.74 \times 10^{-7} < G_{\text{bare}} < 2.00 \times 10^{-7}$ in order to achieve highly sensitive detection of DNA immobilization.

Figure 5 shows typical $I$–$V$ graphs obtained from SWNT network devices after immobilization of probe DNA and hybridization with target DNA. Fifteen network devices, spanning a conductance range of $0.83 \times 10^{-7} < G_{\text{bare}} < 1.20 \times 10^{-7}$, were used to obtain these data. To evaluate this label-free detection method, unlabeled probe DNAs that have sequences that are complementary (P1) and noncomplementary (P2) to target DNAs were covalently immobilized on the network devices. Upon the addition of the target DNA, the complementary probe DNA resulted in a large change in the electrical response shown by a further decrease of the slope in the $I$–$V$ plots (Figure 5a). On the contrary, non-cDNA, to which the target DNA failed to bind, led to little electrical change (Figure 5b). In order to verify that the negligible electrical response from the non-cDNA linked network was truly caused by the absence of DNA hybridization, a negative control experiment was conducted by using the probe alone without the target DNA. No significant change in the electrical signal was observed (Figure 5c), which is similar to that observed with non-cDNA. Thus, it is clear that target DNA hybridization with the non-cDNAs does not occur on the device.

The observed changes in electrical conductance seem to be caused by negative charges associated with the DNA phosphate groups on the SWNT film. It is known that anionic carboxylate groups of nanotubes act as scattering points for electron transport. Thus, negative charges present in DNA, when covalently bonded to SWNTs, can cause an increase in the scattering strength, leading to a decrease in conductance. A subsequent decrease in the conductance is expected after hybridization takes place with target DNA since more anionic phosphate groups were added to the SWNTs. In contrast, non-cDNA on the SWNT films does not interact with target DNA and consequently, there are no change in the number of anionic groups and, thus, the conductance is almost maintained. Further studies are underway to understand the effect of DNA charges on the electrical transport of nanotube network.

For quantitatively estimating the change of electrical response, electrosignal ($G/G_{\text{bare}} - 1$) showing the conductance change after DNA immobilization and hybridization was calculated. As shown in Figure 6, reduction in signal was observed after DNA immobilization, which is consistent with the results of Figure 5. After hybridization of target DNAs, an averaged signal value of SWNT network devices with complementary probe DNA is apparently larger than that observed when non-cDNA is bound.
Thus, these devices display good selectivity in detecting cDNA hybridization.

IV. Conclusion

In this study, the effects of SWNT network density on the performance of label-free electrical detection of DNA hybridization were elucidated. Fluorescence analysis clearly showed that covalent binding between DNA and the carboxylic acid functionality on SWNTs is an effective immobilization method on the SWNT network film. It was found that there exists an optimum range of SWNT network density, having a specific range of conductance for the effective detection of DNA immobilization. The SWNT devices with optimal network densities showed distinctive electrical signals for the detection of interaction by cDNA, as compared with that by non-cDNA immobilization. It was found that there exists an optimum range of SWNT network density, having a specific functionality on SWNTs is an effective immobilization method by the vacuum filtration method, and FTIR spectra of SWNTs, schematic of SWNT film fabrication shortened SWNT obtained by acid treatment, Raman spectra of as-prepared SWNTs, and DNA-attached SWNT film. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgment. This research was supported by the National Research Laboratory Program (R32-2008-000-10142-0), and the Center for Nanoscale Mechatronics & Manufacturing (08K140100414, CNMM).

Supporting Information Available: TEM image of a shortened SWNT obtained by acid treatment, Raman spectra of as-prepared SWNTs, schematic of SWNT film fabrication by the vacuum filtration method, and FTIR spectra of SWNT film and DNA-attached SWNT film. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


JP09080564