Carbon nanotube interfaces for single molecular level bio sensing

H. Vedala*, S. Roy*, T-H. Kim*, W.B. Choi*

*Department of Mechanical and Materials Engineering, Florida International University, Miami, FL 33174, USA, choiw@fiu.edu

ABSTRACT

Single molecule detection, aimed both at fundamental investigation and applications, has recently been attracting a lot of attentions. The present set of studies is focused on the design, fabrication and optimization of novel carbon nanotube (CNT) probes for recognition of biomolecules (target species) with the accuracy down to molecular level. The sensor platforms are fabricated with aligned grown CNTs on desired substrates with high level of control. We have demonstrated excellent electrochemical sensing of functionalized array of CNT for quantitative and selective detection of a range of metabolites including cholesterol, ascorbic acid and uric acid, in buffer solution as well as in human plasma and blood. In addition, a label-free detection scheme for DNA hybridization as well as environmental gas has recently been demonstrated (Nano Letters vol 8, 26 2008). In this presentation the charge transport of bio-molecular binding at the CNT-transducer will be presented and discussed.

Keywords: Carbon Nanotube, DNA, Hybridization, electric conductance, bio sensors

1 INTRODUCTION

Electronic detection of biomolecules at single molecular level has several advantages as compared to detection of ensemble of molecules. Single molecules studies unravel the intrinsic properties of these molecules which are essential for both fundamental studies and various technological applications [1]. Most of the current available single molecule detection techniques use spectroscopic properties of the molecule which require optical labeling. This adds more complexity to the spectroscopic detection systems. On the other hand use of electronic detection techniques based on nanomaterials provide a direct and label free alternative method.

In this communication we report the development of a novel nanoelectronic platform for measuring direct electrical transport in single-molecule DNA of genomic significance. We have used single-walled carbon nanotube (SWNT) electrodes for anchoring a DNA molecule of compatible diameter (1-2 nm). Characterization of DNA using carbon nanotubes (CNT) has been pursued in the past, motivated by the prospects of CNT as a unique electrode material[2,3]. A couple of recent reports detailed the techniques of creating a nanogap in a SWNT and bridging the gap by organic molecules[4,5]. The preset study extends this concept to overcome the challenge of anchoring and electrically characterizing single-molecule DNA. We also report the influence of local environmental factors such as counterion variation, pH, temperature, ionic strength on charge transport (CT) of double-stranded (ds) DNA molecule at single molecule-level.

2 EXPERIMENTAL

2.1 Nanoelectrode fabrication

SWNTs were synthesized by chemical vapor deposition technique and were suspended in isopropyl alcohol by ultrasonication. Initially a 2 µl droplet of SWNT suspension was spun on an thermally oxidized (500 nm) silicon substrate having photolithographically patterned microelectrodes and bonding pad [6,7]. Electrical contacts to individual SWNT were made by first locating them with respect to prepatterned index marks using field emission scanning electron microscope (FESEM) imaging. That was followed by making contact leads using e-beam lithography and sputtering of 50 nm of Au on 10 nm Ti adhesion layer. To fabricate a pair of nanoelectrodes, Focused Ion Beam (FIB) was used for etching near the center of an individual SWNT segment between the metal electrodes. FIB etching parameters (beam current, exposure time) were optimized to obtain a uniform gap in accordance with the length of the DNA strands.

2.2 Electrode functionalization and DNA attachment

Electrical conductivity of an 80 base–pair (bp) in denatured (ssDNA) and hybridized (dsDNA) form (contour length ~27nm), encoding a portion of the *H5N1* gene of avian *Influenza A* virus (AIV) was measured. The template strand obtained with amine modifications at the 5′ and 3′ ends was hybridized with the unmodified complementary strand at 90°C for 5 min in 10 mM NaAc buffer (pH 5.8) at equimolar concentrations. To measure the electrical conductivity of the dsDNA molecules, the SWNT nanoelectrodes were first functionalized with COOH groups to form a strong covalent bond with amine terminated DNA molecule. This was performed by chemical oxidation of SWNT as reported in [8]. In short the SWNTs were treated with HNO_3 for 1 hour followed by rinsing with DI water and vacuum drying. The sample was then incubated for 30 min in 2 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 5

mM *N*-hydroxysuccinimide (NHS) to convert carboxyl groups to amine-reactive Sulfo-NHS esters. Amine terminated dsDNA molecules from a diluted solution (10 nM) were deposited on the electrodes and a.c. dielectrophoresis technique was used to align and immobilize DNA molecule between the electrodes. The devices were then washed with corresponding buffer solutions to remove non specifically attached DNA molecules. The samples were then blow dried with nitrogen stream.

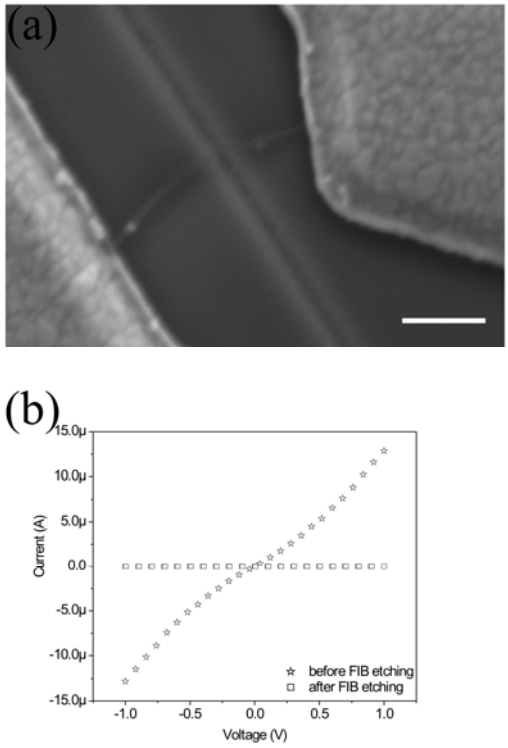

Figure 1: (a) SEM image of a pair of FIB-etched SWNT electrodes connected by Ti/Au micro-contact leads. Scale bar = 100 nm. (b) Room temperature *I-V* characteristics of SWNT before and after FIB etching (30 nm gap)

3 ELECTRICAL CHARACTERIZATION OF SINGLE DNA MOLECULE

3.1 Nanoelectrode electrical characterization

Figure 1 (a) illustrates the SEM image of a single SWNT nanoelectrodes with a gap of 27 nm. Figure 1 (b) depicts *I-V* characteristics of a typical SWNT before and after the etching process. The SWNT exhibited resistance in the range of kΩ. After FIB etching the current decreased from several micro-amperes to a few femto-amperes (noise range of instrument) indicating the nanogap formation. FIB etching also resulted in the formation of a trench in the oxide layer beneath the gap. The presence of the nanotrench (width: 27±3 nm) helps in minimizing the interaction between the anchored DNA molecule and the substrate surface, which, otherwise causes a strong compression deformation of the immobilized DNA molecule and hence a perturbation of CT through it.

As the DNA molecules are coiled in an aqueous medium due to thermal agitation, a strong electric field gradient is essential to straighten and attach them between the electrodes. We used a.c. dielectrophoresis to trap and align the DNA molecule between the SWNT nanoelectrodes with a peak-to-peak voltage of 0.1-1 V and for a frequency range between 0.01-10 MHz. The applied field (40 MV/m) is sufficiently high to overcome Brownian motion, which is dominant in nanoscale objects [9]. Figure 2 shows the AFM image of the DNA molecule attached between the nanoelectrodes after dielectrophoresis.

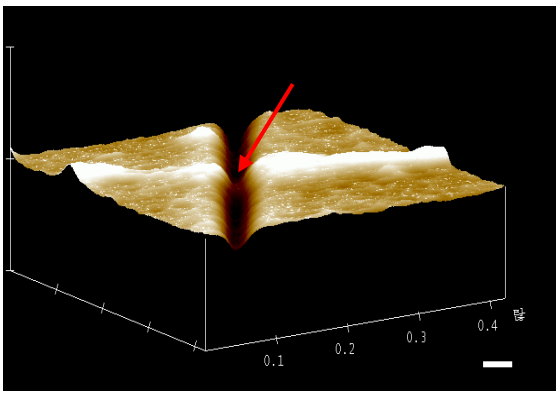

Figure 2: High resolution AFM image of the DNA molecule between the SWNT electrodes. Scale bar = 30 nm.

3.2 Electrical conductivity of single DNA molecule

The I-V characteristics of the double helix, hybridized in NaAc buffer, dried and measured at ambient and in high vacuum (10^{-5} Torr) conditions is shown in Figure 3 (a). At ambient conditions a current signal of 30 pA for a 1 V bias was observed while at high vacuum condition the signal decreased by 33 %. This decrease may be ascribed to the partial removal of water molecules from the proximity of the DNA. In fact, in ambient condition the proton-transfer process in the hydration layer surrounding the DNA promotes the electrical conductivity but diminishes in high vacuum [10].

NSTI-Nanotech 2008, www.nsti.org, ISBN 978-1-4200-8503-7 Vol. 1

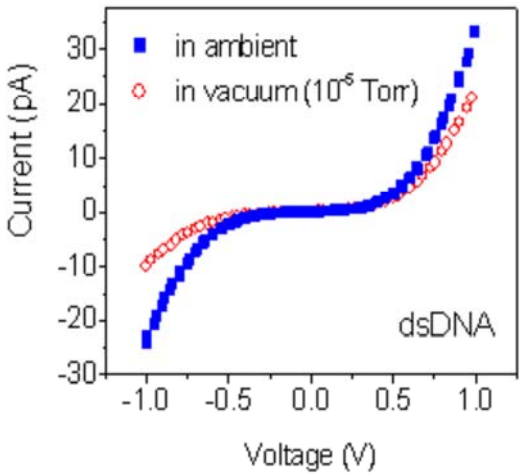

Figure 3: High resolution AFM image of the DNA molecule between the SWNT electrodes. Scale bar = 30 nm.

3.3 Influence of environmental factors on DNA conductivity

The structural and electronic properties of DNA molecule are significantly effect by its local environment. Several earlier reports have shown the strong influence of humidity on the conductivity of DNA molecules. However other environmental factors such as counterion variation, pH and temperature which have substantial effect on DNA conductivity were not studied in detail. The effect of ionic variation on the electrical conductivity of dry DNA molecule was studied by measuring I-V characteristics of dsDNA previously suspended either in sodium acetate (NaAc) or Tris(hydroxymethyl) aminomethane (TE). Figure 4 shows a comparison of I-V characteristics of hybridized DNA (in dry state) previously suspended either in NaAc or TE buffer.

A current signal in the range of 25-50 pA (at 1 V bias) was observed for the DNA molecule in the case of sodium acetate buffer. A nonlinear I-V characteristic was observed indicating a semiconducting behavior of the trapped DNA fragment encoding a specific gene but devoid of any periodic arrangement of the base pairs. In comparison, we observed that dsDNA in TE buffer exhibit almost two orders of magnitude higher current. To further investigate the cause of differences in magnitude, control experiments for both the buffer solutions were performed. A comparison of I-V characteristics after spotting a droplet of DNA-free NaAc and TE buffer, followed by drying in nitrogen stream showed that after drying, TE buffer exhibits about two orders of magnitude higher current as compared to that for sodium acetate. This observation is in accordance reports wherein a large current was observed for a similar control experiment with TE buffer[10]. Hence it is apparent that, unlike sodium acetate, the intrinsic conductivity of TE buffer strongly influences the measured conductivity of DNA and thus can be misled as the intrinsic conductivity of the dsDNA even in the dry state.

Figure 5 (a) shows the influence of pH variation on the conductivity of the DNA molecule. After the I-V measurement of DNA molecule at pH 5.8, the substrates were washed with NaAc buffer (10 mM) with pH values from 3.5 to 9.3 using the same experimental process mentioned earlier. It can be observed that as the pH is increased there is gradual increase in current signal. After measuring conductivity at pH 9.3, I-V measurements were repeated by washing the devices with descending values of pH. Current signals were similar to the values obtained for the corresponding pH values during the increase of pH. A similar trend in pH dependence of DNA conductivity was reported by Lee et al [11]. Further studies have to be performed to understand the true mechanism of pH influence on DNA conductivity.

Figure 5 (b) depicts the influence of variation of temperature on the conductivity of the dsDNA molecule attached between the nanoelectrodes. As the temperature was increased from 25°C (in high vacuum) the current signal decreased gradually. We believe that eventual evaporation of water molecules from the hydration shell surrounding the DNA molecule, and subsequent change in DNA conformation became predominant factors in this case. Further increase in temperature above the melting temperature (T_M = 75.6°C) resulted in complete loss of signal possibly due to the thermal denaturation of DNA. I-V measurements after cooling the device to room temperature yielded current in the range of few pA (≤10 pA) confirming the above assumption.

The influence of ionic strength of buffer on DNA conductivity was also studied. It was found that for buffer concentrations between 10 mM and 100 mM, the current signal varied from 40 - 100 pA. However at high salt concentration (>100 mM) there was a drastic increase in current signal. This could be attributed to the condensation of salt residue on DNA, which might have not been removed by the washing procedure followed in this work. On the other hand at very low ion concentration (<1 mM) the current signal diminished to few pA ranges indicating probable denaturation of dsDNA.

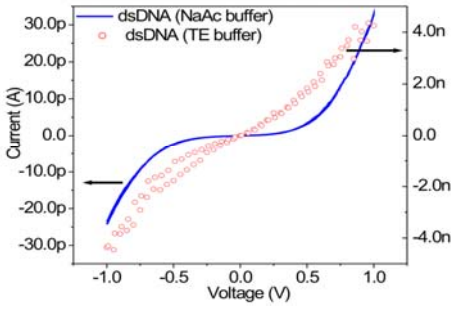

Figure 4 : *I-V* characteristics of a dsDNA molecule previously suspended either in NaAc and TE buffer. The two orders of higher magnitude in current signal observed for DNA in TE is attributed to the high intrinsic conductivity of the buffer

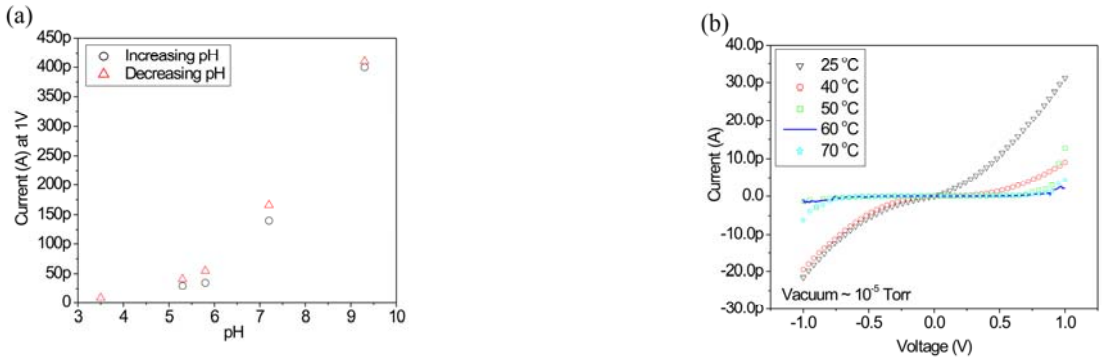

Figure 5 : (a) Effect of pH variation on conductivity of DNA measured at 1V bias. *I-V* of the same device washed with buffer solution of different pH values is shown both for gradual increase and decrease in pH. (b) Temperature effect on DNA conductivity. A gradual increase in the device temperature resulted in a diminishing current signal. Above the melting temperature of the DNA molecule (75.6 °C), there was no detectable signal. (c) Effect of ionic strength (NaAc) variation on DNA conductivity measured at 1V bias. At very low ionic strength (<1 mM), the signal diminished.

To confirm that the current signal was from the immobilized DNA molecule the following control experiment was performed. The devices were treated with DNase I enzyme (37°C, 30 min), followed by cleaning with NaAc and DI water. DNAse I enzyme cuts the DNA regardless of sequence. I –V measurements taken after the cleaning step showed no current signal (noise in fA range) indicating that the current signal obtained earlier was indeed from the DNA molecule bridging the SWNT electrodes.

4 CONCLUSION

In conclusion, we have developed a novel platform based on SWNT nanoelectrodes for directly probing the dc conductivity in DNA at the single molecule level. A n application of DEP in our system causes stretching of the DNA molecules and positioning them between the electrodes. Statistically, for majority of the devices, we observed a current value in the range of 25-40 pA when a dsDNA molecule bridges the SWNT electrodes. Influence of various local environmental factors on DNA conductivity was investigated. A reversible shift in the current signal was observed for pH variation. Influence of ionic strength on DNA conductivity was significant especially at very high (<100 mM) concentrations due to the condensation of salt residues. The present study demonstrates that SWNT can be employed as efficient nanoelectrodes for direct measurements of charge transport in DNA at the single molecule level.

REFERENCES

[1] Singh-Zocchi M, Dixit S, Ivanov V and Zocchi G 2003 *Proceedings of the National Academy of Sciences* 100 7605

[2] Roy S, Vedala H and Choi W 2006 *Nanotechnology* 17 S14-S18

[3] Vedala H, Huang J, Zhou X Y, Kim G, Roy S and Choi W B 2006 *Applied Surface Science* 252 7987

[4] Qi P, Javey A, Rolandi M, Wang Q, Yenilmez E and Dai H 2004 *J Am Chem Soc* 126 11774

[5] Guo X, et al. 2006 *Science* 311 356

[6] Kim D H, Huang J, Shin H K, Roy S and Choi W 2006 *Nano Lett.* 6 2821

[7] Roy S, et al. 2008 *Nano Lett.* 8 26

[8] Chen J, Hamon M A, Hu H, Chen Y, Rao A M, Eklund P C and Haddon R C 1998 *Science* 282 95

[9] Tuukkanen S, Toppari J J, Kuzyk A, Hirviniemi L, Hytonen V P, Ihalainen T and Torma P 2006 *Nano Lett.* 6 1339

[10] Kleine-Ostmann T, Jordens C, Baaske K, Weimann T, de Angelis M H and Koch M 2006 *Applied Physics Letters* 88 102102

[11] Lee J M, Ahn S K, Kim K S, Lee Y and Roh Y 2006 *Thin Solid Films* 515 818