DNA-Functionalized Single-Walled Carbon Nanotube-Based Sensor Array for Breath Analysis

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***Abstract*—The possibility of routine monitoring of metabolic disorders via breath analysis has attracted considerable scientific and clinical interest for many years. The volatile organic compounds (VOCs) in exhaled breath, which are mainly blood borne, particularly provide valuable information about the subject's physiological and pathophysiological conditions. Additionally, it is non-invasive, real-time, painless and agreeable to patients. We have developed a wireless sensor array based on single-stranded DNA (ssDNA)-decorated single-walled carbon nanotubes (SWNT) for the detection of some physiological indicators in breath. Four DNA sequences were used to functionalize SWNT sensors to detect trace amount of methanol, benzene, acetonitrile, dimethyl sulfide, hydrogen sulfide, and acetone, which are indicators of heavy smoking, excessive drinking, and diseases such as lung cancer and diabetes. Our tests indicated that DNA functionalized SWNT sensors exhibit great selectivity, sensitivity, reproducibility, and repeatability. Furthermore, different molecules can be distinguished through pattern recognition enabled by this sensor array. Thus, it has demonstrated a very high potential to be applied in chemical or bimolecular detection for disease diagnostics and health monitoring.**

***Index Terms*—DNA-SWNT sensor, wireless sensor array, breath analysis, pattern recognition**

I. Introduction

Breath provides insight into the physiological and pathophysiological processes in patients' bodies, e.g. the sweet smell of acetone accompanies diabetes [1]-[3]. Breath analysis, as a diagnostic technique, is non-invasive, painless, agreeable to patients, achievable in real time, and can even provide information beyond conventional analysis of blood and urine [4], [5]. Many different analytical techniques were used to analyze exhaled breath, such as gas chromatography and mass spectrometry (GC and MS) [6], [7]. However, they require laboratory setting, significant processing time, expensive instrumentation, and highly trained professionals. Consequently, it cannot be used for individual health monitoring at home or other daily activities. Our goal is to develop a portable, real-time, accurate, easy to use, and cost effective device for breath analysis.

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SWNT, with their specific electrical, mechanical, chemical, and thermal properties, are widely utilized in chemical/biological sensors [8] and agents for drug delivery [9], [10]. Decorating SWNT with a self-assembled monolayer of ssDNA has integrated the selective odorant interactions of ssDNA [11] with the sensitivity of SWNTs to the changes of its surface electronic environment when exposed to analytes [12]. Moreover, the response of these devices to a particular chemical of interest can be optimized by changing the base sequence of the ssDNA.

Exhaled breath consists of oxygen, nitrogen, carbon dioxide, water, inert gases and trace amounts of more than 200 different VOCs. In order to recognize certain molecules in breath, a sensor array of different DNA-decorated SWNT sensors is required and pattern recognition method is preferred to distinguish different chemicals.

Here we introduce a wireless sensor array with six channels to measure the responses of the six DNA-SWNT sensors simultaneously when exposed to different gases [13]. Various DNA decorated SWNT sensors respond differently to different gases. Thus, this real-time wireless sensor array generates a specific pattern for one particular gas, which can be utilized to recognize certain chemicals.

Seven chemicals were selected: 1) water, the common component in breath, 2) methanol, a possible indicator for excessive drinking [14], [15], 3) benzene, a marker that at high levels related with heavy smoking [16], 4) acetonitrile, which is also related with smoking [17], 5) dimethyl sulfide, a potential indicator for lung cancer [18], 6) hydrogen sulfide, a probable indicator of bad breath and 7) acetone, an acknowledged biomarker for diabetes [1]-[3].

II. Experiments

A. Fabrication of ssDNA Decorated SWNT Sensor Array

First, microelectrodes with a 3μm gap were fabricated by photolithography followed by E-beam depositing a Cr/Au (20nm/150nm) layer onto a silicon oxide substrate (Fig. 1 (1)-Fig. 1(4)). Then SWNT (diameter: 1~2nm;

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length: 2~5μm, Brewer Science Company) were assembled between the microelectrodes, just as bridges (Fig. 1 (5)), via solution-based DEP assembly. An AC (Alternating Current) signal of 1Vpp and 10MHz frequency was applied between the electrodes after the placement of 2μL of the dispersed SWNT solution onto the top of the electrode gap. After aligning SWNT between the microelectrodes, DNA were assembled on SWNT through π-π stacking (Fig. 1. (6)). DNA sequences are shown below:

DNA 24GT: GTGTGTGTGTGTGTGTGTGTGTGT
DNA 24A: AAAAAAAAAAAAAAAAAAAAAAAA
DNA 24Aa: Amine-AAAAAAAAAAAAAAAAAAAAAAAA-Amine
DNA 24Ma: Amine-GACCTGTTCAAGGACCTGTTCAAG-Amine

B. *Wireless Sensing Package*

The major advantage of our wireless nanosensor array is that it provides a flexible and universal platform to approach a wide variety of applications (Fig. 2a). For example, the signal conditioning module can always be reprogrammed and adjusted, allowing for the integration of diverse sensors into this wireless sensing platform. For the actual system (Fig. 2b), the nanosensor array module is on the top layer, the signal conditioner is on the middle one, while the bottom layer contains low power microcontroller board called Waspmote board and a RF module.

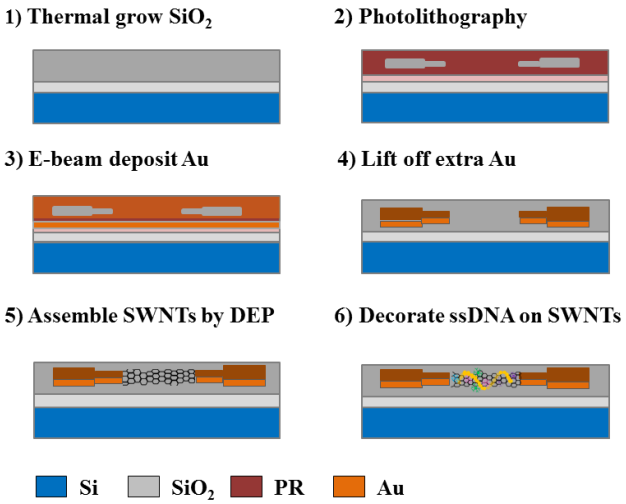

Figure 1. Fabrication process for DNA functionalized SWNTs on microelectrodes

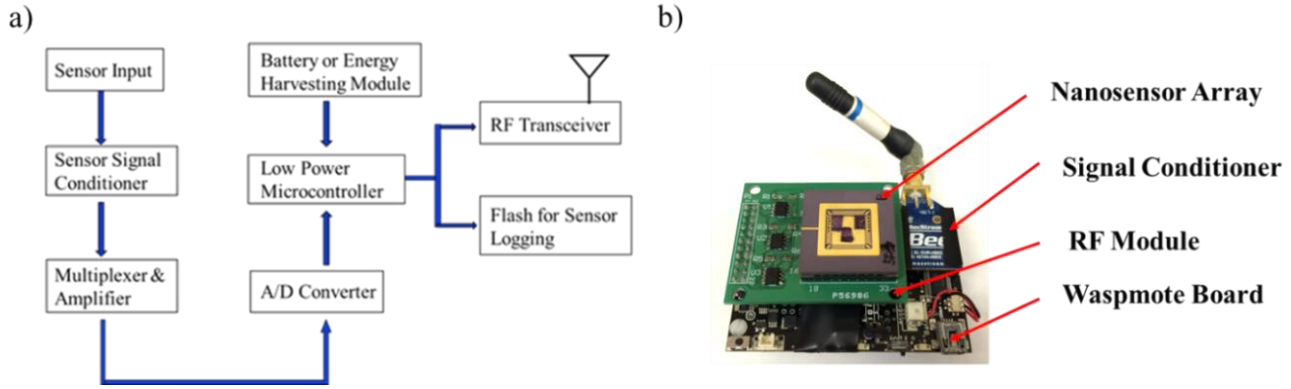

Figure 2. a) The wireless sensor node functional block diagram for the wireless sensor node; b) photograph of the wireless nanosensor array system

C. *Sensing Strategies and Characteristics*

The data collected was wirelessly transmitted to a Waspmote gateway, which was attached to a PC via a USB port. Resistance changes of six different DNA-SWNT sensors when exposed to analytes were real-time monitored, plotted simultaneously, and stored for further analysis.

D. *Chemicals Detection by Sensor Array*

Water, methanol, acetone, acetonitrile, benzene and DMS vapors were generated from their solutions at room temperature. The concentrations of acetone and DMS were modified by adding dipropylene glycol (DPG), which didn't cause any response to SWNT [19]. The equilibrium vapor pressures of water, methanol, benzene, acetonitrile, acetone and DMS at 20 ℃ were 18.66, 97.66, 70, 71, 0.038 and 0.076 torr respectively. H2S was generated from the reaction between FeS and H2SO4 and the vapor pressure was estimated to be 0.027 torr (36ppm). DNA 24GT, DNA 24A, DNA 24Aa, and DNA 24Ma were decorated on SWNT sensors and their responses to these vapors were measured.

III. Results and Discussions

In this assembly, a large number of individual nanotubes bridged the microelectrodes. The majority of the nanotubes assembled were successfully aligned between the two microelectrodes (Fig. 3a). The tiny white dots, believed to be aggregated ssDNA molecules, were coated onto the SWNT bundles through π-π stacking.

Five different nanosensors decorated with the same DNA sequence were used to detect each chemical. The resistance changes after exposure to the chemical vapors for 10 minutes were recorded (Fig. 3b).

Water, methanol, acetone, and acetonitrile are polar molecules and hydrophilic. Benzene is a nonpolar organic molecule with very limited solubility in water (hydrophobic). Hydrogen sulfide and dimethyl sulfide

(DMS) are polar molecules but hydrophobic. For methanol and water, which are polar molecules with similar structure, the responses had the same trend (the red and black curves in Fig. 3b). However, since methanol is more volatile and has a higher vapor pressure at room temperature, the resistance changes for methanol (the red curve) are higher than that for water (the black curve). The sensor array's response to acetonitrile was similar to methanol, since acetonitrile was also a polar molecule and hydrophilic, but was much smaller compared to that for methanol. It is because the nitrile group (C≡N) is less hydrophilic than the hydroxyl group (O-H). Therefore, the affinity of DNA to acetonitrile is less strong than it to methanol. For acetone, a hydrophilic polar molecule, the pattern of response was similar to water and methanol's, but the resistance changes were much smaller resulting from a lower polarity and weaker hydrophilic property due to the carboxyl group (C=O) and two methyl groups. DNA decorated nanosensors barely responded to benzene and DMS (the green and blue curves). It is because benzene and DMS are hydrophobic molecules which do not tend to adsorb on the DNA decorated SWNTs. Especially for benzene, DNA decorated SWNT sensors exhibited even smaller responses compared to bare SWNT sensors. For hydrogen sulfide, the response pattern was different from all the others. The resistance of SWNT sensor decorated with DNA 24GT decreased significantly when exposed to hydrogen sulfide. However, the resistances of the other nanosensors all slightly increased when exposed to hydrogen sulfide. It is very likely that the interaction between nucleobases G and/or T with free thiol group (-SH) is much stronger than that of nucleobase A and C. It can be due to the highly polarizable divalent sulfur centers in hydrogen sulfide. This unique response of the DNA 24GT decorated SWNT sensor to hydrogen sulfide can be used to differentiate it from other vapors. Study of the concentration and temperature effects is in progress and will better demonstrate our sensor array's high selectivity and sensitivity.

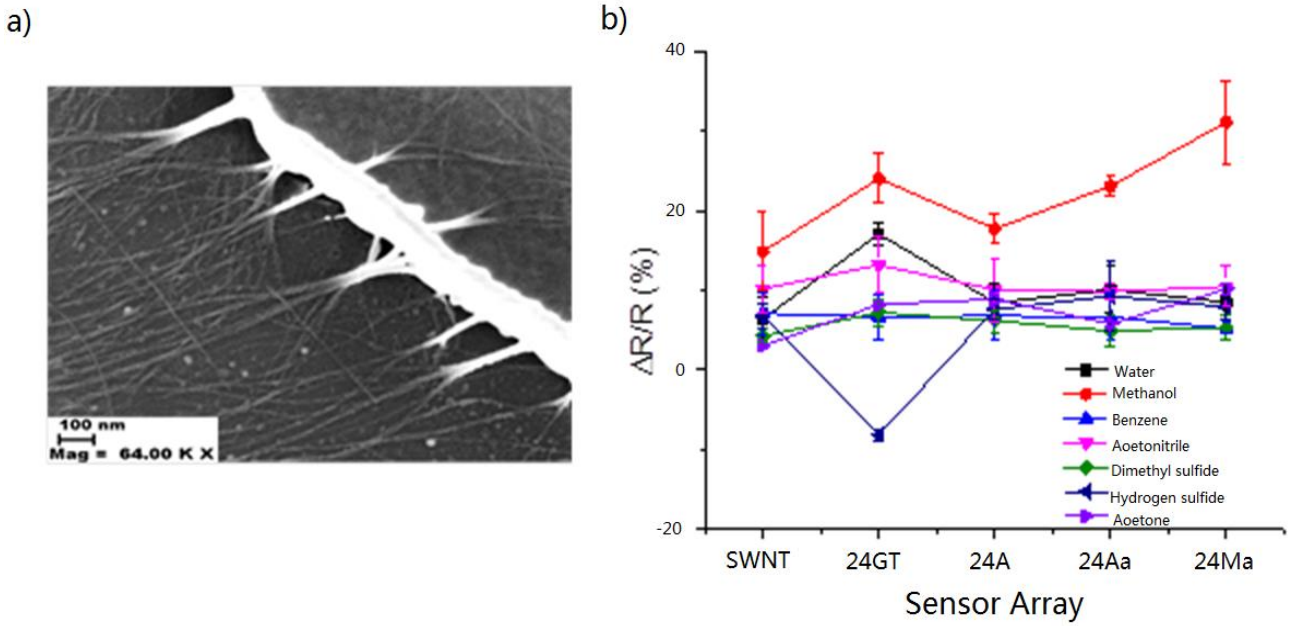

Figure 3. a) SEM image of ssDNA-decorated SWNTs assembled between microelectrodes; b) resistance changes of DNA 24GT, DNA 24A, DNA 24Aa and DNA 24Ma-fucntionalized SWNT nanosensors and of bare SWNT when exposed to water, methanol, benzene, acetonitrile, dimethyl sulfide, hydrogen sulfide and acetone vapors. Error bars= ± standard deviation and n=5

IV. Conclusion

We have developed a wireless nanosensor array based on ssDNA decorated SWNT on micro devices. The DNA functionalized SWNT sensors presented reversible and repeatable changes in response to different vapors. The nanosensor array, decorated with four different DNA sequences, was tested with seven vapors which can indicate the physiological and pathophysiological conditions of individuals. The results indicated that DNA increased the affinity of SWNTs to hydrophilic molecules, which was due to the surface properties of SWNTs being altered from hydrophobic to hydrophilic by the DNA decoration. In addition, the DNA 24GT decorated SWNT sensor exhibited a different behavior (decrease in its resistance) compared to other types of SWNT sensors when exposed to hydrogen sulfide. This showed a great potential to distinguish hydrogen sulfide from other vapors by DNA 24GT decorated SWNT sensor. Additionally, measuring the responses from six different DNA functionalized SWNT sensors simultaneously and analyzing the response pattern will allow one to selectively detect different analytes. This array-based sensing approach provides high selectivity, good sensitivity, great repeatability and excellent precision for gas analysis. It reveals high potential for real-time and highly sensitive breath analysis for non-invasive disease diagnostics and health monitoring.

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