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Noninvasive Disease Diagnosis through Breath Analysis Using DNA-Functionalized SWNT Sensor Array

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***Abstract*—**Noninvasive diagnostics of diseases via breath analysis has attracted considerable scientific and clinical interest for many years and become more and more promising with the rapid advancements in nanotechnology and biotechnology. The volatile organic compounds (VOCs) in exhaled breath, which are mainly blood borne, particularly provide highly valuable information about individuals' physiological and pathophysiological conditions. Additionally, breath analysis is noninvasive, real-time, painless, and agreeable to patients. We have developed a wireless sensor array based on single-stranded DNA (ssDNA)-functionalized single-walled carbon nanotubes (SWNT) for the detection of a number of physiological indicators in breath. Seven DNA sequences were used to functionalize SWNT sensors to detect trace amount of methanol, benzene, dimethyl sulfide, hydrogen sulfide, acetone, and ethanol, which are indicators of heavy smoking, excessive drinking, and diseases such as lung cancer, breast cancer, and diabetes. Our test results indicated that DNA functionalized SWNT sensors exhibit great selectivity, sensitivity, and repeatability; and different molecules can be distinguished through pattern recognition enabled by this sensor array. Furthermore, the experimental sensing results are consistent with the Molecular Dynamics simulated ssDNA-molecular target interaction rankings. Thus, the DNA-SWNT sensor array has great potential to be applied in chemical or biomolecular detection for the noninvasive diagnostics of diseases and personal health monitoring.

***Keywords*—**Breath analysis, DNA-SWNT sensor array, diagnosis, noninvasive.

I. Introduction

BREATH provides insights 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 standard laboratory setting, significant processing time, expensive instrumentation, and highly trained professionals. Consequently, it cannot be used for individual health monitoring at home or during daily activities. Our goal is to develop a portable, accurate, easy to use, real-time, and cost effective device for breath analysis.

SWNTs, with their specific electrical, mechanical, chemical, and thermal properties, are widely utilized in chemical/biological sensors [8] or as agents for drug delivery [9], [10]. However, a major disadvantage of SWNT sensors is the lack of sensing specificity. To solve this problem, an effective scheme to functionalize the SWNT sensors is required to enable them to specifically respond to a variety of molecular targets. Modification of SWNTs with polymers [11]-[13] and biomolecular complexes [14]-[16] has shown great enhancement in its specificity and sensitivity. Among these molecules, DNA can nonspecifically bind to the sidewalls of SWNTs through hydrophobic interactions, π-π bonding [17], and possibly amino-affinity. A system that consists of SWNTs decorated with a self-assembled monolayer of ssDNA has integrated the selective odorant interactions of ssDNA [18] with the sensitivity of SWNTs to the changes in its surface electronic environment when exposed to analytes [19]. Moreover, the response of these devices to a particular molecule of interest can always be optimized by changing the base sequence of the ssDNA. As a result, functionalization of SWNTs with DNA has demonstrated attractive prospects in various fields including the detection of molecular targets, solubilization in aqueous media, the nucleic acid sensing, and probing biomolecular interactions [15], [20]-[22]. Furthermore, a number of different ssDNA-functionalized SWNT sensors can be integrated into a wireless sensor array on one micro device to detect/distinguish different targets or biomarkers simultaneously [23]-[25]. An array-based sensing approach is enormously efficient in real-time, highly sensitive and fast detection due to its high selectivity, good sensitivity, great repeatability and excellent precision.

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.

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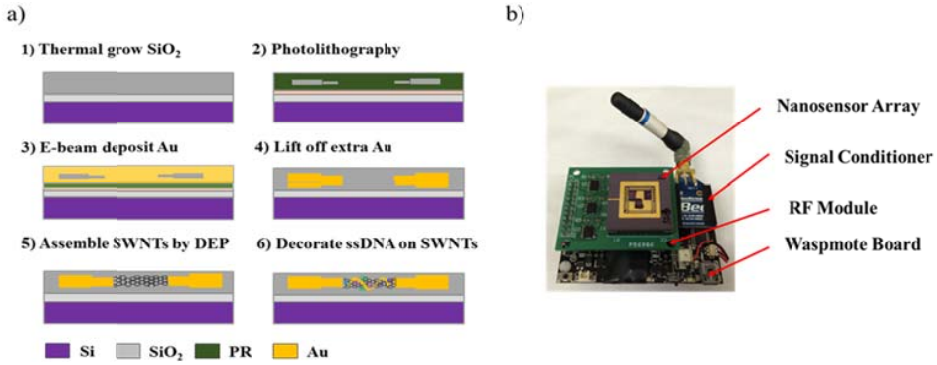

Fig. 1 (a) Fabrication process of DNA-SWNT sensors, (b) Illustration of the wireless nanosensor array system

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 [23]. 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 molecular targets.

Six molecular targets were selected: (1) methanol, a possible indicator for excessive drinking [26], [27], (2) benzene, a marker that at high levels related with heavy smoking [28], (3) dimethyl sulfide, a potential indicator for lung cancer [29], (4) hydrogen sulfide, a probable indicator of bad breath, (5) acetone and (6) ethanol, acknowledged biomarkers for diabetes [1]-[3].

II. Methods

The sensing system includes the DNA functionalized SWNT sensor array and a wireless sensing package. First, microfabrication process was applied to fabricate ssDNA-SWNT sensor chips (Fig. 1 (a)). Microelectrodes with a 3 μm gap were fabricated by photolithography followed by E-beam depositing a Cr/Au (20 nm/150 nm) layer onto a silicon oxide substrate (Fig. 1 (a) (1)-(4)). Then SWNT (diameter: 1~2nm; length: 2~5μm, Brewer Science Company) were assembled between the microelectrodes, just as bridges (Fig. 1 (a) (5)), via solution-based DEP assembly. An AC (Alternating Current) signal of $1V_{pp}$ 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 (a) (6)). DNA sequences assembled are listed below:

- DNA 24A: AAAAAAAAAAAAAAAAAAAAAAAA
- DNA 24Aa: Amine-AAAAAAAAAAAAAAAAAAAAAAA-Amine
- DNA 24G: GGGGGGGGGGGGGGGGGGGGGGGG
- DNA 24GT: GTGTGTGTGTGTGTGTGTGTGTGT
- DNA 24T: TTTTTTTTTTTTTTTTTTTTTTTT
- DNA 24Ma: Amine-
- GACCTGTTCAAGGACCTGTTCAAG-Amine
- DNA 24C: CCCCCCCCCCCCCCCCCCCCCCCC

Our wireless nanosensing system includes the nanosensor array module on the top layer, signal conditioner on the middle one, and low power microcontroller board called Waspmote board and a RF module at the bottom layer (Fig. 1 (b)). 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.

Methanol, benzene, dimethyl sulfide, acetone, and ethanol vapors were generated from their solutions at room temperature. The concentrations of acetone and dimethyl sulfide were modified by adding dipropylene glycol (DPG), which didn't cause any response to SWNT [30]. The equilibrium vapor pressures of methanol, benzene, ethanol, acetone, and dimethyl sulfide at 20^0C were 97.7, 70, 44.6, 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 24A, DNA 24Aa, DNA 24G, DNA 24GT, DNA 24 T, DNA 24Ma and DNA 24C were decorated on SWNT sensors and their responses to these vapors were measured.

III. Results and Discussion

In this sensor assembly, a great number of individual nanotubes bridged the microelectrodes. The majority of the nanotubes assembled were successfully aligned between the two microelectrodes (Fig. 2). The tiny white dots, believed to be aggregated ssDNA molecules, were functionalized onto the SWNT bundles through π-π stacking.

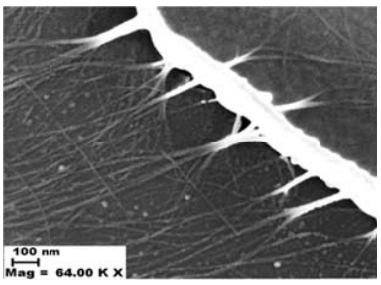

Fig. 2 SEM image of ssDNA-decorated SWNTs assembled between microelectrodes

Three separate 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. 3).

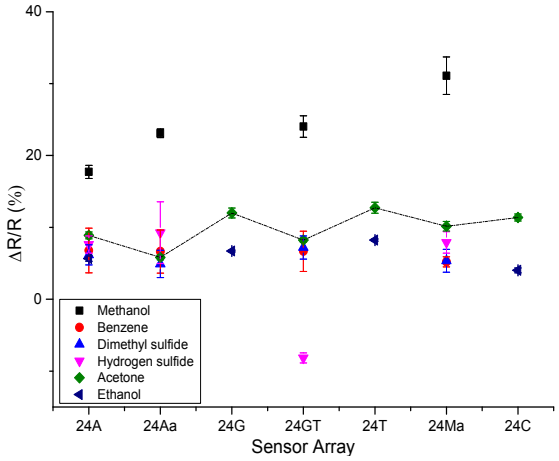

Fig. 3 Resistance changes of DNA 24A, DNA 24Aa, DNA 24G, DNA 24GT, DNA 24T, DNA 24Ma, and DNA 24C-functionalized SWNT nanosensors when exposed to methanol, benzene, dimethyl sulfide, hydrogen sulfide, acetone and ethanol vapors. Error bars = ± standard deviation and n = 3

Methanol, acetone, and ethanol are polar molecules and hydrophilic. Benzene is a nonpolar organic molecule with very limited solubility in water (hydrophobic). Hydrogen sulfide and dimethyl sulfide are polar molecules but hydrophobic. The reaction between these targeted molecules and DNA-SWNT sensor is highly sequence dependent, thus, different molecules can be distinguished through pattern recognition enabled by this sensor array, for example, acetone (line in Fig. 3). For acetone, a hydrophilic polar molecule, the pattern of response was similar to methanol's, but the resistance changes were much smaller resulting from a lower polarity and weaker hydrophilic property due to the carbonyl group (C=O) and two methyl groups. DNA decorated nanosensors barely responded to benzene and dimethyl sulfide. It is because benzene and dimethyl sulfide are hydrophobic molecules which do not tend to adsorb on the DNA decorated SWNTs. For hydrogen sulfide, the response pattern was different from all the others. The resistance of SWNT sensor functionalized 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. The sensing results of acetone and ethanol, especially by the DNA 24A, DNA 24G, DNA 24C and DNA 24T, are in great agreement with the Molecular Dynamics simulated results elsewhere [31]. Study of the concentration and temperature effects is in progress and will better demonstrate our sensor array's high selectivity and sensitivity.

IV. Conclusion

We have developed a wireless nanosensor array based on ssDNA functionalized SWNTs on a micro device. The DNA functionalized SWNT sensors presented reversible and repeatable changes in response to different vapors. The experimental sensing results are also consistent with the Molecular Dynamics simulated ssDNA-molecular target interaction rankings indicating the reliability of computational simulation on DNA sequence selection. The nanosensor array, decorated with seven different DNA sequences, was tested with six vapors indicating individuals' physiological and pathophysiological conditions. DNA increased the affinity of SWNTs to hydrophilic molecules due to the surface properties of SWNTs being altered from hydrophobic to hydrophilic by the DNA decoration. In addition, 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. Measuring responses from seven different DNA functionalized SWNT sensors simultaneously and analyzing the response pattern will allow one to selectively detect various molecular targets. This array-based sensing approach provides high selectivity, good sensitivity, and great repeatability for breath analysis. Using bottom-up computational approaches, like Molecular Dynamics simulation, and applying DNA as a tunable biomaterial, this DNA array technology would enable highly sensitive breath analysis for non-invasive disease diagnostics and personal health monitoring.

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