Screening and discrimination of Hepatocellular carcinoma patients by testing exhaled breath with smart devices using composite polymer/carbon nanotube gas sensors

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Abstract—There is currently great interest in the medical application of electronic nose and chemical sensors, especially in the area of early diagnosis and screening of diseases. In this study, a pocket electronic nose based on eight nanocomposite gas sensors made of polymer and functionalized single-walled carbon nanotubes (f-SWCNTs) was shown to be capable to discriminate between the two sample groups of hepatocellular carcinoma (HCC) patients and healthy control subjects. Polymer/f-SWCNTs sensor-integrated electronic nose system has been designed and fabricated to be suitable for exhaled breath detection. This chemical gas sensor array has a good sensitivity to a broad range of volatile organic compounds (VOCs), sufficiently to cover the chemical species contained in the human exhaled breath such as acetone, ammonia, methyl-ethyl-ketone, and toluene (excluding water that has negligible impact on sensitivity of the sensors). The obtained results demonstrate that the e-nose has a potential to discriminate the patterns of exhaled breath odor from five healthy controls from five HCC patients, as analyzed by the principal component analysis (PCA) with 95% of the confidence level. In the near future, this approach may become very useful in clinical application to serve as a non-invasive device for screening patients with early-stage liver cancer.

Keywords—Liver cancer monitoring; Electronic nose; Human exhaled breath; Gas sensors; VOCs.

I. INTRODUCTION

Volatile biomarkers detection is a potential screening method that has received much attention from biomedical researchers. Breath analysis devices are increasingly being tested for diagnosing various diseases such as diabetes [1], cancer [2], influenza virus aerosols in human exhaled breath [3], Alzheimer’s and Parkinson’s disease [4]. There are a large number of volatile compounds in human breath that are caused by metabolism in the human body and these released into the air in the exhaled breath. So far, odor detection has been performed using traditional analytical techniques such as selected ion flow tube mass spectrometry (SIFT-MS) [5], solid phase micro-extraction (SPME) and gas chromatography with mass spectroscopy or GCMS [6].

Cancer diagnosis based on exhaled breath analysis is conducted under the hypothesis that cancerous cells generate some specific biomarker compounds associated with cancer, or a change in pattern in the intensities of common VOC’s. Recent reports have shown that odor detection by dogs can successfully diagnose certain cancers, such as lung cancer [7-8], breast cancer [9], bladder cancer [10], and gastric cancer [11].

![Fig. 1. The schematic diagram of VOCs exhaled breaths monitoring for liver cancer screening method.](image-url)
Therefore, a new scanning technology for cancer biomarkers which is convenient and inexpensive would be extremely beneficial for the early diagnosis and screening of cancers, and would help to reduce the number of cancer deaths. In the case of liver cancer (hepatocellular carcinoma, HCC), its incidence is noticeably increasing in many countries, and is one of the top five causes of cancer death worldwide. One of the major factors in the high mortality in patients with HCC is the fact that the medical equipment used for early diagnosis and screening of cancer at an early-stage is not optimal. Conventional diagnostic techniques use alpha-fetoprotein (AFP) blood levels, ultrasound, CT scans and MRI scans are used to screen for and diagnose this cancer [12]. However, all of these diagnostic methods are not always affordable for routine examination, both for hospitals in developing countries and also for poor patients. In addition, they are time-consuming and may require highly trained staff to interpret. Therefore, researchers around the world are actively trying to find other alternative methods to overcome these drawbacks by testing chemical sensors and electronic nose (e-nose) [13]. The development of novel e-nose instruments to identify well-established cancer biomarkers has been steadily increasing over the last decade. The ability to detect volatile markers from the liver cancer using e-nose depends on the sensitive and selective sensing materials. Thus, several types of sensing materials and methods for supporting e-nose system have been proposed such as conductive polymer [14], metal oxide [15] and nanocomposite materials [16].

In this paper, we present a new alternative approach for cancer diagnosis based on the detection of breath volatiles using e-nose. Our e-nose system comprised of an array of chemoresistive gas sensors based on nanocomposites of polymer and functionalized single walled carbon nanotubes (SWCNTs). Such approach can be illustrated by a schematic diagram as shown in Fig. 1. It is hoped that the analysis of exhaled breath fingerprints and volatile biomarkers for liver cancer using multivariate analysis methods together with gas sensor arrays will provide some information which would be very significant for future applications in biomedical engineering.

II. EXPERIMENTAL DETAILS

A. Fabrication of Composite-Polymer Gas Sensor Arrays

In this work, we fabricated eight different types of nanocomposite materials between polymer and functionalized single-walled carbon nanotubes (SWCNTs) as a gas sensor array for a pocket electronic nose intended to detect exhaled breath odors as shown in Table I.

The two-step fabrication process for gas sensors is described below. Firstly, the composite solutions were prepared by dissolving each polymer (3 mg) in 1 mL of the proper solvent and then sonicated for 15 minutes to obtain the homogeneous solution of mixture. After that, the homogeneous solutions were added to functionalized SWCNTs (Loading of 15 wt% SWCNT), stirred (15 minutes) and then sonicated (30 minutes) continuously.

Polymer/f-SWCNTs solution was deposited over the interdigitated finger electrode structure (IDES) by spin-coating technique at 1500 rpm spin speed for 30 seconds to form a composite thin film on the substrate; the electrical resistivity is approximately 1-35 kΩ. Finally, the polymer/f-SWCNTs composites thin film were annealed in the oven at temperatures of 100°C for 3 hours in order to optimize and restructure the electrical conducting network and remove residual solvents.

Nanocomposite materials from polymer and functionalized single-walled carbon nanotubes form complicated interconnected networks that act as electrical pathways. Changes in electrical resistance of these gas sensors depends on the physical and chemical adsorption of the volatile compounds onto/into the polymer and carbon nanotube networks, causing them to swell and change in the electrical resistance (see Fig. 2)

B. Subject and Collection of Exhaled Breath

In our experiment, exhaled breath samples were divided into two groups in order to achieve reliable results in recognition and classification of the odors. Group A were patients with known hepatocellular carcinoma (HCC) disease, diagnosed according to internationally accepted criteria; (5 males), aged between 35-60 years. Group B were healthy controls without any chronic disease conditions; (5 males), aged between 60-69 years whose personal records were screened to exclude any significant disease. This clinical study involved using human volunteers and was conducted with the approval of the ethics committee at Ramathibodi Hospital, Mahidol University. All patients and volunteers signed a consent form before entering the study.

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**TABLE I. INFORMATION OF COMPOSITE POLYMER/CARBON NANOTUBE GAS SENSORS FOR EXHALED BREATH DETECTION.**

<table>
<thead>
<tr>
<th>Code</th>
<th>Polymer</th>
<th>Functionalized SWCNTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Poly (styreneacetaldehyde) partial isobuty/methyl mixed ester (PSE)</td>
<td>SWCNTs-OH</td>
</tr>
<tr>
<td>C2</td>
<td>Poly (styreneacetaldehyde) partial isobuty/methyl mixed ester (PSE)</td>
<td>SWCNTs-COOH</td>
</tr>
<tr>
<td>C3</td>
<td>Polyvinylpyrrolidion (PVP)</td>
<td>SWCNTs-COOH</td>
</tr>
<tr>
<td>C4</td>
<td>Polyvinyl chloride (PVC)</td>
<td>SWCNTs-OH</td>
</tr>
<tr>
<td>C5</td>
<td>Polystyrene sulfonic acid</td>
<td>SWCNTs-COOH</td>
</tr>
<tr>
<td>C6</td>
<td>Polyvinyl chloride (PVC)</td>
<td>SWCNTs-COOH</td>
</tr>
<tr>
<td>C7</td>
<td>Polyvinyl alcohol (PVA)</td>
<td>SWCNTs-COOH</td>
</tr>
<tr>
<td>C8</td>
<td>Polyvinyl chloride (PVC)</td>
<td>SWCNTs-NH2</td>
</tr>
</tbody>
</table>

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Fig. 2. The response behavior of polymer/f-SWCNTs composite gas sensors when odorant molecules percolate through the sensing layer.
Collection of exhaled breath odor was performed by breathing air directly into glass vials which were stored prior to analysis. This method is known as breath sampling for offline analysis. Each volunteer was trained in the correct method for exhaled breath collection into a container. The exhaled breath odor was collected in the morning at least one hour after consuming any food or tooth brushing in order to eliminate the food odor contamination or bacteria in the mouth. Moreover, each sample was tested within an hour of collection to prevent any ageing of exhaled breath odor to distort the results.

C. VOCs & Exhaled Breath Odor Measurement System

The performance of nanocomposite gas sensor array was evaluated by two measurement systems, namely a static measurement and a dynamic measurement. The first system called the static system, measures the sensing response of the sensors to some selected volatile organic compounds (ammonia, toluene, acetone, methyl ethyl ketone and water). This step is very important as it defines the capability of each sensor to respond to these VOCs. The initial baseline is recorded for 2 minutes after which VOCs (concentrations as 20 ppm, 40 ppm and 60 ppm) is injected into the chamber using micro-syringe for 10 minutes.

The dynamic measurement which is the second system performs as a working system of artificial nose, in which exhaled and inhaled breathing are mimicked by switching the flow of nitrogen gas (reference gas) and the sample gas into a chamber of the gas sensors. In this set-up the reference gas and sample gas have been stored and allowed to flow from two separate bottles. Solenoid valves are used for switching between the reference and sample gas. Nitrogen gas was allowed to flow first to the sensing chamber for 6 minutes to obtain a steady baseline resistance and then switched to allow flow from the sample bottle for 30 seconds; this constitutes one complete cycle. The measurement was conducted for 6 cycles and repeated 3 times each. The flow rate of nitrogen gas was 100 mL/min.

The signals from both systems was acquired via a DAQ card USB which recorded the change of electrical resistances in real time and visualized it on a computer screen via a user interface software programmed under LabVIEW program.

III. RESULTS AND DISCUSSIONS

A. The Sensing Response Analysis

The sensitivity of gas sensor (R_r) was studied by calculating the changing electrical resistance of nanocomposite films upon interaction with VOCs at room temperature. In principle, when the sensing material interacts with VOCs, the polymer matrix starts to swell leading to an increase in the distance between SWCNTs networks. This can be interpreted on the computer screen and seen as increase in resistance on exposure to the odor gas.

Thus, R_r (%) was calculated as percentage change in electrical resistance when odor molecules percolate through the sensors (R_s) over the initial resistance baseline (R_i) (see Fig. 3) as written in the equation below.

\[
R_r(\%) = \left( \frac{R_s - R_i}{R_i} \right) \times 100 = \left( \frac{\Delta R}{R_i} \right) \times 100
\]  

B. Characteristics of Nanocomposite Gas Sensors

In this part, we shall discuss the response of each sensor upon exposure to VOCs from exhaled breath. Normal composition of human breath contains inorganic and organic compounds such as water, CO2 and ammonia whose concentration will differ in the event of pathological cases [20]. But some compounds such as ethyl methyl ketone, acetone and toluene were found especially in the breath of HCC patients. Therefore, in this work, we tested the volatiles found particularly in the exhaled breath using a static gas measurement system to achieve the optimal detection and sensor response. Five volatiles (acetone, ammonia, methyl ethyl ketone, toluene and water) were used as target gases for exploring the performance of the gas sensors. Fig.4 shows the plot of sensing responses with five volatiles as a function of time. The results demonstrate that our nanocomposite gas sensors yield linear response to the analyte concentration over the concentration range of 20-60 ppm. Overall, we observed highest response to ammonia and the second highest response to toluene. Sensor C3 shows good response to all VOCs. Sensor C5 behaves like C3 except for ammonia and acetone. Sensor C6 specifically shows an excellent response to ammonia. In addition, all sensors show the lowest response to water.

The behavior of composite sensors with VOCs can be explained by the capability of nanocomposite sensors to interact with analytes. When the analytic molecules adhere to the surface of polymer through dipole-dipole or van der Waals interactions, they start to swell and cause change in the conducting path of SWCNTs network resulting in the change of the sensor resistance. The percentage response of each sensor depends firstly on how easily the analyte gas diffuses or interacts with the structure of polymer and secondly on the type of functionalized group of SWCNTs.
Therefore, we have found out that all polymer/SWCNTs gas sensors from this work can be used for detecting various volatiles in the exhaled breath.

Fig. 5 shows the plot of sensor resistance when exposed to exhaled breath from HCC patients as measured under the dynamic gas sensing measurement system. Fig. 6 shows a comparison of average percentage response of each sensor when tested with exhaled breath in two groups. The results show that most gas sensors show higher sensing response to the exhaled breath of HCC patients than to healthy controls except for C5 sensor.

Fig. 7. Principal components analysis (PCA) plot in two-dimensional projection (PC1-PC3) between the exhaled breath from HCC patients and healthy controls.


C. Principal Component Analysis

A pattern recognition method was applied to analyze the sensor array response data. The max/min values of the data were used as selected features which were further processed by the principal component analysis (PCA) method in order to reduce the dimension of the data and eliminate noise. The PCA is a mathematical method that uses data from each sensor in which the number of measurements were arrayed into a matrix format. The feature in the analysis is used to calculate the covariance matrix and values of the eigenvector.

PCA in two dimensions (PC1-PC3) of the data obtained from HCC and healthy subjects is shown in Fig.7. It was shown that both sample groups were well separated according to the first principal component (PC1) that explained 86.2%, and the third principal component (PC3) that explained only 2.9% of the variation. This implies that both samples have highly correlated features (having high contents of volatiles in common). So the results indicate that there is a distinct difference between the volatile pattern of HCC patients and the healthy controls.

IV. CONCLUSIONS

Polymer/functionalized-SWCNT nanocomposites were fabricated as gas sensor array for use in an electronic nose system. The sensors were capable of detecting volatile vapors in exhaled breath aimed to discriminate HCC patients from healthy subjects. The results have revealed that sensor C3 and C5, which are polyvinyl-pyrrolidone/SWCNTs-COOH and polystyrene sulfonic acid/SWCNTs-COOH, respectively, showed good response to the exhaled volatiles. In addition, sensor C6 (polyvinyl chloride (PVC)/SWCNTs-COOH) responded well to ammonia. We tried to classify HCC patients and healthy controls using PCA pattern recognition. It was distinctly seen that the polymer/SWCNT nanocomposite sensor array successfully discriminated HCC patients from the healthy persons. The obtained results might open new opportunities in clinical diagnosis for early scanning of liver cancer with time and cost advantages.

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