Detection of Mixed Volatile Organic Compounds and Lung Cancer Breaths Using Chemiresistor Arrays with Crosslinked Nanoparticle Thin Films

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ABSTRACT: The ability to create sensing thin films for chemiresistors using crosslinked nanoparticle thin films with subtle structural differences in terms of interparticle linker molecular structure, nanoparticle composition and size is important not only for tuning sensitivity and selectivity in constructing a sensor array but also for enhancing stability of the sensors under ambient sensing conditions. In this report, we show that arrays of chemiresistors with such subtle structural differences are viable for detecting mixed volatile organic compounds (VOCs) and breath biomarkers under ambient conditions. The sensor arrays exhibit nanostructure-tunable sensitivity to VOCs and mixtures, with a limit of detection as low as 20-ppb easily achievable for acetone. Preliminary testing of the sensor array in detecting breath samples from limited lung cancer patients, which consists of certain mixed VOCs as biomarkers, has also demonstrated the capability of breath recognition of lung cancer patients from healthy individuals under ambient sensing conditions. The recognition statistics were analyzed, showing the potential viability of achieving the desired sensitivity, selectivity, and accuracy in the breath sensing, the implication of which is discussed in terms of optimization of the sensor arrays for potential lung cancer screening.

Key words: Breath sensor; lung cancer; chemiresistor array; pattern recognition; nanostructured sensing materials; point-of-care medical device.
1. Introduction

There have been increasing interests in exploring molecularly-capped nanoparticles as sensing materials on chemiresistors for the detection of volatile organic compounds (VOCs). [1-10] In particular, the coupling of chemiresistor arrays with pattern recognition techniques has been demonstrated to be powerful for addressing some of the challenging issues in chemical sensing of VOCs [7-11]. This capability is very important for developing breath sensors or electronic noses with great potentials in disease detection. In comparison with GC or GC-MS based breath analysis, breath sensors have the advantages of portability, cost-effectiveness, easy operation, fast reading, and the potential for chemical fingerprinting when an array of sensors is coupled with pattern recognition. Recent studies of nanoparticle-based chemiresistor arrays have shown potentials for detecting cancers, diabetes, and other diseases [12-24]. For detecting lung cancer [14,20-22], most of the routine techniques are often invasive, expensive and slow, and require complex instruments and pre-concentration of biomarkers[14,20]. In contrast, breath sensing is a fast, non-invasive, and low-cost diagnostic method that relates certain VOCs in exhaled breath to medical conditions. Technically, there are still challenging issues in developing breath sensors for diagnostic applications. One of the challenging issues is the variation in the VOC profiles and/or concentrations between the different studies in terms of VOCs in lung cancer because of the lack of normalization and standardization. As documented in a recent review, about 36 VOCs are considered to be most reproducible and validated VOCs related to lung cancer, among which there are seven families, including hydrocarbons, primary and secondary alcohols, aldehydes and branched aldehydes, ketones, esters, nitriles, and aromatic compounds [21]. To achieve the desired sensitivity and selectivity to the VOCs, it is essential to use different sensing array materials. There have been examples of nanoparticles [12-14,16-19,23], polymers [15] and carbon blacks [15,20]. The coupling of sensor arrays with the pattern recognition methods is also essential for the breath recognition between healthy people and lung cancer patients. Haick and co-workers [14,20-21] have demonstrated the viability of casting nanoparticle thin films on chemiresistor arrays for detection of breath VOCs of lung cancer patients, and further substantiated the usefulness of breath VOCs for detecting lung cancer in the general population as documented earlier by Phillips and co-workers using GC-MS method [22].

In this report, we describe findings of an investigation of molecularly-linked gold nanoparticles that are self-assembled or printed on chemiresistor arrays for detection of mixed VOCs and breath samples from lung cancer patients. This work has expanded our earlier work [18] by constructing sensor arrays using molecularly-linked nanoparticles as sensing thin films on both rigid and flexible chemiresistors prepared by self-assembly or printing method. In addition
to the capability of recognition of mixed VOCs, we have demonstrated the ppb-level detection capability of our chemiresistor arrays with the nanostructured sensing thin films. We have also demonstrated that the sensor arrays are capable of distinguishing the breath samples of lung cancer patients from those of healthy individuals under ambient sensing environment. This study was motivated by the recent report on the possibility of profiling genetic mutations of lung cancer cells based on the detection of patterns of VOCs emitted from cell membranes by Haick et al. [14,24]. In comparison with the detection methods reported previously, our sensor array system doesn’t require a vacuum system and pre-concentration, which is important for portable devices in lung cancer screening, and uses crosslinked nanoparticle thin films as sensing materials, which is important for enhancing stability of the sensors under ambient conditions. In an in-vitro study [24], a volatile fingerprinting assay for genetic mutations in cancer cells identified five VOCs using an array of sensors that are associated with the oncogenes in terms of mutations including the epidermal growth factor receptor (EGFR), and fusion of the echinoderm microtubule-associated protein-like 4 gene to the anaplastic lymphoma kinase (ALK) gene. EGFR is the cell-surface receptor, and some lung cancer tumor cells have a DNA mutation that affects the EGFR, known as EGFR mutation-positive. EGFRs mutated lung cancer has an increased rate of uncontrolled tumor growth, which can speed up the cancer’s progression. An ALK mutation is an abnormality in a gene originally identified in lung cancer cells, and its mutation testing positive is known as ALK positive. Indeed, ion mobility spectrometric analysis of breath VOCs from lung cancer patients with and without EGFR mutation [25] recently showed that patients positive for EGFR mutation displayed a significantly higher n-dodecane than that of those negative. N-dodecane analysis was demonstrated to be useful to discriminate the EGFR mutation. Such findings constitute a motivation for our study, aiming at exploring our molecularly-mediated thin film arrays for exploring the potential viability of breath screening. Note that the use of molecularly-linked nanoparticle thin films, in contrast to use of thin films of nanoparticles formed by simple casting and evaporation in most of previous studies of VOCs and cancer breaths, has not been reported for chemiresistive recognition of mixed VOCs and cancer breaths under ambient conditions. The results in this report represent the first demonstration of such thin films formed by interparticle linkages such as hydrogen-bonding and alkyl chains as sensing arrays on chemiresistors in detection of binary and ternary mixed VOCs and in a preliminary test of breath samples for lung cancer patients.

2. Material and methods

Hydrogen tetrachloroaurate trihydrate (HAuCl₄•3H₂O, 99%), tetraoctylammonium bromide (TOA⁺Br⁻, 99%), decanethiol (DT, 96%), sodium borohydride (NaBH₄, 99%), 11-mercaptoundecanoic acid (MUA), 16-Mercaptohexadecanoic acid (MHA, 90%), 3-mercaptopropanoic acid (MPA, 99%) and alkyl di thiols (ADT, HS-(CH₂)ₙ-SH), including 1,3-propanedithiol (PrDT, 99%), 1,4-butanedithiol (BDT, 97%), 1,5-pentanedithiol (PDT, 96%), 1,6-Hexanedithiol (HDT, 96%) were purchased from Aldrich and used as received. Solvents such as hexane (99.9%) and toluene (99.8%) were from Fisher, and ethanol (99.9%) and acetone (99.9%) from Aldrich. Water was purified with a Millipore Milli-Q water system.

Gold nanoparticles of 2 nm diameters (2.0 ± 0.7 nm, Au₂nm) encapsulated with DT monolayer shells were synthesized by two-phase reduction of AuCl₄⁻ using Brust’s method [26] with a synthetic modification [27]. Details for the synthesis were previously described. [27,28] Gold nanoparticles with larger sizes (5.2 ± 0.5 nm, Au₅nm) were synthesized by a thermally-activated processing route [27,28]. Briefly, a measured amount of the as-synthesized nanoparticle solution was heated to 145~150°C. The products, washed by ethanol and separated by centrifuge, were re-dispersed in hexane. Details for the morphology and size distribution can be found in our previous reports [27,28].

The preparation of the thin films followed the one-step exchange-crosslinking-precipitation method [8-10,29]. For an ADT linked thin film, for example, it involved immersion of a chemiresistor device into a mixture of hexane solution of DT-capped Auₙnm (30 μM) and ADT (HS-(CH₂)ₙ-SH, 50 mM) for the thin film assembly. The nanoparticle to mediator ratios were controlled, typically about 1:50-500 (~1:50 for PrDT, 1:500 for BDT, PDT) for Au₂nm, and about 1:400-4000 (~1:400 for PrDT, 1:4000 for PDT) for larger-sized particles. Typical film thickness ranged from 100 to 300 nm [8-10,29].

2.2. Construction of sensor arrays.

Sensor arrays are designed to create subtle differences of individual sensors in sensitivity and selectivity to the targeted VOCs by manipulating the detailed nanostructures of the sensing thin film and microelectrode parameters, [10, 18] which are used to produce a set of reliable and unique profiles for analysis using pattern recognition techniques. Sensors in the array differ from each other in terms of the chemical and physical properties of the interparticle linking molecules (e.g., hydrophobicity, hydrogen-bonding capacity, chain length, aromatic structure, etc.), the particle composition or size, and the parameters of microelectrodes (e.g., gaps). The interparticle linkages involve hydrogen-bonding structures via head-head hydrogen bonding of carboxylic acid groups at the head group of alkyl thiols or alkyl chain structures via α,ω-alkyl dithiols, details of which were described in our previous reports [8-9]. Because of the subtle differences in capping
and linking structures and particle sizes, their conductivity properties differ from each other, and their response profiles are different upon exposures to different analytes.

The chemiresistor devices featured patterned interdigitated microelectrodes (IMEs) on glass or PET substrates [8-10,29]. For a photo-lithographically fabricated IME device on glass substrates, the microelectrode pattern typically consists of 100 pairs of gold microelectrodes of 200 μm length, 10 μm width, 100 nm thickness, and 5 μm spacing. Such patterns were also fabricated with copper microelectrodes on PET substrates. In addition, devices with simple microelectrode patterns on PET substrates were also made by nanoink-printing and laser-sintering processes. One typical example of such a device was a pair of serpentine AuCu electrodes of 30 cm length, 90 μm width, and 100 μm gap on a PET substrate [30].

Sensor arrays were constructed assembling or printing nanoparticle thin films on the chemiresistor devices. The nanoparticle thin films were prepared by either self-assembly via exchange-corsslinking-precipitation method (sa-) [8-10, 27-29] or printing (pr-) (e.g., screen printing or inkjet printing) using pre-linked nanoparticle inks [30, 31]. For example, Array I was constructed from combinations of pr-MUA-Au_{2nm}/IME (1), sa-MPA-Au_{2nm}/IME (2), pr-MPA-Au_{2nm}/IME (3), sa-MHA-Au_{2nm}/IME (4), and pr-MUA-Au_{2nm}/IME* (5, *- Note that this device was serpentine-type microelectrodes fabricated by laser sintered and nanoink-printed microelectrodes on PET substrate [30]). Array II was constructed from combinations of sa-NDT-Au_{2nm} (1), sa-HDT-Au_{2nm} (2), sa-PDT-Au_{2nm} (3), and sa-BDT-Au_{2nm} (4). Sensor Array III was constructed from combinations of sa-MUA-Au_{7nm} (1), sa-BDT-Au_{2nm} (2), and sa-NDT-Au_{2nm} (3).

2.3 Generation of ppb-level VOCs.

VOCs in the range of ppm (V) level concentrations were generated by gas flow and mixing method. The gas flow was controlled by an array of calibrated Aalborg mass-flow controllers (AFC-2600), details of which were previously described. [8-10] A ppb-level VOC was prepared by two steps. The first step was to quantitatively introduce μL-quantity of pure acetone into a large sealed jar (20 L) purged with N₂, in which acetone is completely vaporized, yielding a definite concentration (33 ppm). Water also been injected into the sealed jar system, establishing an equilibrium between the gas and solution phases. In the second step, a μL-level of sample was withdrawn from the 33 ppm acetone container, and was injected into the ~5 mL sensor testing chamber, achieving an acetone concentration in the ppb-level inside the test chamber.

2.4 Sensor response measurements.

A custom-designed computer-interfaced multi-channel multimeter was used to measure the resistance of the sensors upon flowing VOCs over the devices. The chemiresistor devices were housed in a Teflon chamber. The test chamber was purged with N₂ and the analyte vapor
alternatively. The vapor concentration (ppm (V)) was determined by partial vapor pressure and mixing ratio in the flow system. All experiments were performed at room temperature, 22 ±1 °C. N₂ (99.99%, Airgas) was used as a reference gas and as a diluent to change vapor concentration, as previously described [8-10,29]. The response sensitivity was based on the relative differential resistance change, ΔR/R, (R is the resistance in response to vapor exposure and Rᵢ is the initial resistance of the film), versus vapor concentration.

2.5 Breath sample collection and detection.

Breath sample were collected with six lung cancer patients, who were all in Stage 4 of non-small-cell lung cancer with measurable disease. The medical information is shown in Table 1.

Table-1 Medical condition of the lung cancer patients

<table>
<thead>
<tr>
<th>Patient-#</th>
<th>EGFR</th>
<th>ALK</th>
<th>Disease Sites</th>
<th>Current Medication</th>
<th>Previous Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-1</td>
<td>+</td>
<td>-</td>
<td>L., B.</td>
<td>Tarceva</td>
<td>Ra., Ch.</td>
</tr>
<tr>
<td>P-2</td>
<td>-</td>
<td>+</td>
<td>L., M., B.</td>
<td></td>
<td>Ch., Anti ALK</td>
</tr>
<tr>
<td>P-3</td>
<td>-</td>
<td>-</td>
<td>L.</td>
<td>Gilotrif</td>
<td>Ch., Anti EGFR</td>
</tr>
<tr>
<td>P-4</td>
<td>-</td>
<td>-</td>
<td>L., M., B.</td>
<td>Nivolumab</td>
<td>Ch., Anti VEGF</td>
</tr>
<tr>
<td>P-5</td>
<td>-</td>
<td>-</td>
<td>L., M.</td>
<td>Navelbine</td>
<td>Ch., Im.</td>
</tr>
<tr>
<td>P-6</td>
<td>-</td>
<td>-</td>
<td>L., M., B.</td>
<td>Taxotere</td>
<td>Ch.</td>
</tr>
</tbody>
</table>

Notes: a) L.: Lung; B.: Bone; M.: Mediastinal. b) Ra.: Radiation; Ch.: Chemotherapy; Im.: Immunotherapy. c) Anti EGFR. d) Immunotherapy. e) Chemotherapy. f) Anti VEGF.

All the patients are positive in adenocarcinoma. EGFR showed positive for P-1 and negative for the rest of the patients. ALK showed positive for P-2 and negative for all others. These patients were asked to have a light breakfast, and the sample was collected 2 hours later using Quintron breath collection bag. The healthy individuals, who are cancer-free, non-COPD (chronic obstructive pulmonary disease), and non-smoking people, were also asked to have light breakfasts for the sample collection. The same methods were used to collect breath samples from the healthy people. All samples were analyzed within two weeks after the sample collection. Typically, 1 mL of breath sample was introduced into the test chamber with a syringe under a N₂ flow at 20 mL/min. For all the breath samples, at least three repeated tests were conducted.

3. Results and Discussion

In this section, we first discuss the sensor response characteristics of the sensor arrays for detection of a number of VOC mixtures directly or indirectly related to lung cancer with controlled concentrations in a nitrogen-flow system. This is followed by the discussion of the limit of detection of the sensor arrays for the detection in both dry and humid environments. Lastly, we describe the results in testing breath samples from healthy people and lung cancer patients to demonstrate the viability of the sensor arrays.
3.1 Detection of Single and Mixed VOCs

In this subsection, we first show data of the sensors and sensor arrays in response to different VOCs and their mixtures to establish the general response characteristics using relatively high vapor concentrations because of the easy variation by our flow control system. Data for low concentration and limit of detection will be described in next subsection. The selected VOC and mixtures are related to the VOCs in human and lung cancer breaths, but not necessarily the exact VOCs reported for lung cancers [21], the variation of which is beyond the scope of this preliminary study in demonstrating potential viability of our arrays for the targeted application.

Fig. 1 shows a representative set of data to illustrate the response profiles of a sensor array (I) in response to acetone (Ac), hexane (Hx) and their mixture (Ac + Hx) in relatively high concentration range. The two sets of curves correspond to the responses to acetone, hexane, and acetone + hexane mixture of different concentrations of a sensor array with two different sensors. The sensor array consists of two sensing films: pr-MUA-Au_{2nm} and sa-MPA-Au_{2nm}. First of all, the MUA-Au_{2nm} exhibits a higher response than MPA-Au_{2nm} with the VOCs. Secondly, for the MUA-Au_{2nm}, it shows higher response to acetone than hexane, whereas, MPA-Au_{2nm} shows opposite trends in terms of acetone and hexane response. The response time reflects vapor adsorption or desorption kinetics, which depend on a combination of the vapor-nanostructure interaction and the vapor diffusion in the thin film which is responsible for the response time.

![Figure 1. Response profiles for two sensor films (Array I: pr-MUA-Au_{2nm}/p-IME (a) and sa-MPA-Au_{2nm} (b)) in response to acetone (Ac; 1.54, 3.08, 4.62, 6.16, 7.70, 9.24, 10.78 (×10^4 ppm)), Hexane (Hx; 1.01, 2.02, 3.03, 4.04, 5.05, 6.06, 7.07 (×10^4 ppm)), and their mixture (Ac + Hx; 1:1 mixing ratio).](image)

PCA (Principle Component Analysis) was used to analyze the data for mixed VOCs, as shown in Fig. 2 for several different mixtures, including acetone and hexane (Fig. 2A), acetone and water (Fig. 2B), and acetone and iso-propanol (Fig. 2C) in a relatively high concentration range controlled by the flow system. As shown by the PCA results from the sensor testing of
acetone, hexane and mixture (Fig. 2A), each of the three groups is well separated from the others in the PC1-PC2 plane. Similar results were also obtained for sensor responses to acetone, water (Wa) and mixture (Ac + Wa) (Fig. 2B). For acetone, iso-propanol (Iso-Pr), and mixtures (Ac + Iso-Pr) with different mixing ratios (Fig. 2C), clear separations are shown. The good separations serve as a promising indication of viability of the sensor array in monitoring VOCs. The sharp contrast of the response profiles between acetone and hexane vapors is remarkable, demonstrating the feasibility of achieving high selectivity.

Figure 2. PCA plots: (A) Plots of acetone (black, Ac; 3 - 10 ($\times$10$^4$ ppm)) and hexane (red, Hx; 2 - 7($\times$10$^4$ ppm)) and their mixture (Ac + Hx; 1:1 mixing ratio) (Array I: sa-MPA-Au$_{2nm}$ and pr-MUA-Au$_{2nm}$). (B) Plots of acetone (black, Ac; 3 - 10 ($\times$10$^4$ ppm)) and water (red, Wa; 3 - 10 ($\times$10$^3$ ppm)) and their mixture (Ac + Wa; 1:1 mixing ratio) (Array I: pr-MPA-Au$_{2nm}$, sa-MHA-Au$_{2nm}$, sa-MPA-Au$_{2nm}$). (C) Plots of acetone (black, Ac; 3 - 10 ($\times$10$^4$ ppm)) and Iso-Propanol (red, Iso-Pr; 2 - 8 ($\times$10$^3$ ppm)) and their mixtures with different mixing ratios (Ac + Iso-Pr; 1:1, green, and Ac + Iso-Pr; 1:2, blue) (Array II: sa-HDT-Au$_{2nm}$, sa-PDT-Au$_{2nm}$, sa-MUA-Au$_{7nm}$, sa-PrDT-Au$_{2nm}$, sa-BDT-Au$_{2nm}$, and sa-NDT-Au$_{2nm}$).

In Fig. 3, PCA results for a number of sets of VOCs and their mixtures are compared in a concentration range controllable by our mass flow system, showing good separations for most VOC mixtures. The slight overlap between acetone, mixture of acetone and ethanol could reflect the hydrophilic characters of ethanol and MUA-Au$_{2nm}$ thin film. In this experiment, hexane, ethanol, iso-propanol, acetone, and benzene were used to represent five types of VOCs, i.e., hydrocarbons, primary and secondary alcohols, ketones, and aromatic compounds [21].
Two important aspects of the above PCA separations must be emphasized. First, the separation of the different mixtures was not due to baseline drifts of the sensors because the separation was achievable under controlled conditions without any drift. Secondly, the separation was not intended for identifying individual VOC, rather to show the viability of sensor array recognition of different mixtures. A further demonstration of the ability to identify individual VOC from a mixture is part of our on-going investigations which involve refining individual sensing thin film’s selectivity in correlation with GC-MS analysis of breath composition, and careful data analysis in relation to optimization of sensors in an array, as done in our earlier work[32], which is needed to address the complexity caused by the different numbers of variables and parameters in PCA analysis.

3.2 Sensitivity and Limit of Detection

To determine the sensor sensitivity, we first examined the response data for vapors in the high concentration range (> 1ppm) that were generated by two flow meters with different flow ratios. Fig. 4 shows acetone sensitivity data for MUA-Au2nm, thin films derived with different film preparation methods, including pr-MUA-Au2nm (red) and sa-MUA-Au2nm (black). For comparison, earlier sensitivity data [19] obtained with an array of self-assembled thin films is shown in Fig. 4. The printed thin film’s sensitivity, 1.5x10⁻⁵ ppm(V)⁻¹, is clearly greater than that for the self-assembled thin film, 4.8x10⁻⁶ ppm(V)⁻¹, the latter is close to the sensitivity reported earlier 1.85x10⁻⁶ ppm(V)⁻¹ (Fig. 4, dashed line).
Figure 4. Response sensitivities to acetone vapor for pr-MUA-Au$_{2nm}$/l-IME (a) (active area: 2.2 cm$^2$) and sa-MUA-Au$_{2nm}$ (b) (active area: 0.6 cm$^2$) device. Dashed line: Plot of response sensitivity of a sensor with sa-MUA-Au$_{2nm}$/IME (active area: 4.0x10$^{-3}$ cm$^2$) in response to acetone vapor [19].

In a separate set of experiments, the sensor response profiles were also compared for sensors with printed MUA-Au$_{2nm}$ thin films of different thicknesses in response to acetone (Fig. 5). The sensors show an increase in response with acetone concentration, while there are some subtle differences in response time. Thinner thin films exhibit shorter response time than thicker ones, consistent the expectation of the vapor partition equilibrium in the film. Fig. 5B shows the sensitivity of the four sensors with different thickness in response to four different vapors, exhibiting different response sensitivities. There is a clear increase in sensitivity when the film thickness increases from 95 to 340 nm, after which the change is insignificant. For hexane and acetone, the thickness dependence of the sensitivity is less significant in comparison with those for toluene and ethanol. Since the sensor sensitivity can be manipulated by tuning the linking molecule, nanoparticle size, or film thickness, considerations of response differences must be taken when comparing response sensitivities.

Figure 5. (A) Sensor response profiles for MUA-Au$_{2nm}$-printed thin films with different thickness (95 (a), 233 (b), 341 (c), and 1000 nm (d)) in response to acetone with different concentration. (B). Plots of the response sensitivities in response to four different vapors: hexane (a), acetone (b), toluene (c), and ethanol (d).

To determine the limit of detection (LOD), the sensor responses to VOCs in the low concentration range were also analyzed. Fig. 6 shows a representative set of data for acetone at ppb-level concentration. The sensor shows negative response with acetone (Fig. 6A), likely due to a subtle difference in the change of dielectric medium properties of the thin films upon vapor sorption [33]. The response exhibits linear response vs. acetone concentration (Fig. 6B).
Figure 6. (A) Sensor responses to four different acetone concentrations: 666 (a), 333 (b), 33 (c), ppb. (B) Plot of sensitivity vs. acetone concentration. Sensor film: sa-NDT-Au$_{2\text{nm}}$.

The resulting sensitivity of $1.53\times 10^{-6}$ ppb$^{-1}$ is 1000 times higher than the response sensitivity obtained in the higher concentration range. This difference is believed to reflect the different extent of molecular interactions of vapor molecules adsorbed in the nanostructured film. There is an increased adsorbate-adsorbate interaction in the film under the higher vapor concentration range in comparison with that under the lower concentration range, which changes the partition coefficient of vapor molecules between vapor and film phases. The LOD was estimated from three times of standard deviation of the noise signal. Based on the noise level of $\Delta R/R_i$ ($2.8\times 10^{-5}$), the estimated LOD was less than 18 ppb for the sensor device used in this experiment.

It is important to note that the responses to water vapor and VOC can be separated in the low concentration range, as shown in Fig. 7 with a array of PDT-Au$_{2\text{nm}}$ and BDT-Au$_{2\text{nm}}$ upon the introduction of acetone vapor saturated with water. Control experiment was also performed for assessing the humidity effect. The sensor responses feature a sharp change in $\Delta R/R_i$ upon exposure to acetone followed by a gradual decrease, and show a higher response to acetone than water. However, BDT-Au$_{2\text{nm}}$ shows both positive responses toward water and acetone vapor, whereas PDT-Au$_{2\text{nm}}$ shows negative response for both water and acetone vapor. The response time is about 2 sec. The sharp contrast in response is remarkable between sensors in the low concentration range, demonstrating the feasibility of achieving high selectivity in recognition between acetone and water vapors, which provides the possibility of separation of lung cancer breath VOCs at low concentrations.
Figure 7. Responses of a sensor array in response to water vapor (Stage 1) and acetone vapor (Stage 2) (Array III: PDT-Au2nm (red, a) and BDT-Au2nm (black, b)). (RH: 90%)

Note also that PCA analysis of the data for pure water vapor and water vapor saturated acetone vapor (data not shown) indicated a good separation. In summary, the sensor array shows clear differences in sensitivity to acetone between high vs. low concentration range, which reflects partially the different time and degree for adsorption equilibrium in the sensing thin films.

3.3 Detection of Breath Samples.

As a preliminary evaluation of the feasibility of the array in recognition of breath samples of lung cancer and healthy individuals, limited breath samples were collected from lung cancer patients, who were in Stage 4 of non-small-cell lung cancer, and tested (see Table 1). Different sensors showed responses differently with lung cancer’s breath samples and healthy people’s breath samples. Based on these response data, an optimized array was identified that consisted of three sensor elements (sa-MUA-Au7nm, sa-BDT-Au2nm, and sa-NDT-Au2nm) with which the response data to breath samples from healthy and lung cancer subjects along with the control experiments were analyzed by PCA.

A representative set of 2-D and 3-D PCA score plots is shown in Fig. 8. These PCA plots showed a good separation of the sensor response data between the healthy human breath and the lung cancer patient breath samples.
As shown in Fig. 9, P-2 and P-4 are falling into one group, while P-3, P-5, and P-6 fall into another group based on the specific point distance from P-1. P-1 appears to be in a single group. For P-3, the data point appears to fall somehow between the first group (2 and 4) and the second group (5 and 6) based on the separation distance. Statistic clustering analysis was also performed with the sensor data (Fig. 9). The sensor data appear to fall into 3 groups based on a statistics clustering method, which involves group observations into clusters based on their distances in terms of cluster variables so that each cluster has homogenous features. The result is quite consistent to the results from the distance grouping method in the PCA data.

Note that P-1 apparently falls into one group, which is consistent with the fact that P-1 is the only one showing EGFR positive. This separation could reflect a significant difference of the VOCs of the exhaled breaths in lung cancer patients between EGFR positive and negative. This finding appears to be consistent with the recent finding based on ion mobility spectrometric analysis of VOCs from the breaths of lung cancer patients with or without EGFR mutation, which
indicated that those with EGFR positive showed significantly higher n-Dodecane than those with EGFR negative [25]. For P-5 and P-6, both of them are receiving chemotherapy treatment, their sensor data seem to indicate that their VOCs could be different from those who do not receive this treatment. Note that the degree of separation is rather limited for the current PCA data, and it was likely influenced by other variables such as dietary differences, personal care product usage, and other environmental factors. The limited number of the lung cancer patients for this test was another issue that certainly needs to be taken into consideration for further investigation.

To further assess the statistic aspect of the data, Discriminant Analysis (DA) was also used to analyze the data. DA is a supervised statistical analysis method, aiming at finding the best possible separation between two previously-known groups. The condition in the analysis is maximal variance between the two groups while maintaining minimal variance between members of the same group. A linear function was used for DA classification, which yields canonical variables (CVs) as its output (Fig. 10A).

![Figure 10. Discriminant analysis results of the sensor data. (A) CV1-CV2 plot for the sensor array data of breath samples between healthy individuals (red circle) and lung cancer patients (black square), which are separated by the vertical dashed line. (B) Classification results.](image)

The result shows a good separation between lung cancer patients and healthy individual’s breath samples. While our lung cancer patients were limited at this time, we could have collected breath samples from more healthy individuals for the data comparison. However, we kept the same number of healthy individuals as the cancer patients for a balanced data analysis for this preliminary study. Nevertheless, the result is further substantiated by the sensitivity, specificity, and accuracy data (Fig. 10B). The result showed a sensitivity of 100.0 %, a specificity of 83.3% and an accuracy of 91.7%. While the breath samples collected from patients were very limited because of the nature of a feasibility study in this work, the findings did provide a promising indication of feasibility for future in-depth investigations. This indication is not affected by confounding factors, as supported by the observation of insignificant differences among breath
samples collected from different genders or under different fasting conditions [18]. This finding is also consistent with an earlier work showing that the statistical analysis is irrespective of age, gender, lifestyle, and other confounding factors [34].

4. Conclusions

Taken together, the nanostructured sensor arrays have shown high sensitivity and selectivity in detecting mixtures of VOCs with a limit of detection as low as 20 ppb. This capability is also shown viable for potentially recognizing breaths from lung cancer patients and healthy individuals under ambient operation conditions. These findings constitute a promising basis of further delineation of the recognition rate through testing more breath samples from lung cancer patients and healthy individuals, which will provide useful information for developing a point-of-care device for breath monitoring of lung cancer patients in the future. This effort will be aided by increasing the population groups for diagnosis and classification purposes with significant confidence levels.

Acknowledgment

Part of the preliminary work performed by August Perez and Yujung Jane Jung is acknowledged. The work was supported by the National Science Foundation (CMMI 1100736), and in part by funds from SUNY Research Collaboration and Network of Excellence.
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