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Detection of mixed volatile organic compounds and lung cancer breaths using chemiresistor arrays with crosslinked nanoparticle thin films

Wei Zhao[a], Lubna F. Al-Nasser[b], Shiyao Shan[a], Jing Li[a], Zakiya Skeete[a], Ning Kang[a], Jin Luo[a], Susan Lu[b], Chuan-Jian Zhong[a,*], Carolyn J. Grausgruber[c], Ronald Harris[c]

[a] Department of Chemistry, State University of New York at Binghamton, Binghamton, NY, 13902, USA
[b] Systems Science and Industrial Engineering, State University of New York at Binghamton, Binghamton, NY, 13902, USA
[c] Broome Oncology, 30 Harrison St., Suite 100, Johnson City, NY, 13790, USA

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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.

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

* Corresponding author.
E-mail address: cjzhong@binghamton.edu (C.-J. Zhong).

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[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 and co-workers [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.

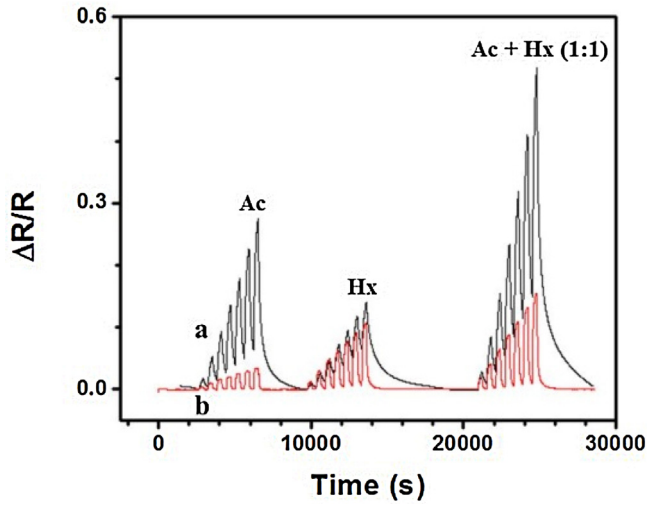

Fig. 1. Response profiles for two sensor films (pr-MUA-$Au_{2\,nm}$/IME (a) and sa-MPA-$Au_{2\,nm}$/IME (b) from Array I) in response to acetone (Ac; 1.54, 3.08, 4.62, 6.16, 7.70, 9.24, 10.78 ($\times 10^4$ ppm)), Hexane (Hx; 1.01,2.02, 3.03, 4.04, 5.05, 6.06, 7.07 ($\times 10^4$ ppm)), and their mixture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

Table 1
Medical condition of the lung cancer patients.

Patient-#	EGFR	ALK	Disease Sites[a]	Current Medication	Previous Treatment[b]
P-1	+	–	L., B.	Tarceva[c]	Ra., Ch.
P-2	–	+	L., M., B.		Ch., Anti ALK
P-3	–	–	L.	Gilotrif[c]	Ch., Anti EGFR
P-4	–	–	L., M., B.	Nivolumab[d]	Ch., Anti VEGF
P-5	–	–	L., M.	Navelbine[e]	Ch., Im.
P-6	–	–	L., M., B.	Taxotere[e] + Cyramza[f]	Ch.

[a] L.: lung; B.: Bone; M.: mediastinal.
[b] Ra.: radiation; Ch.: chemotherapy; Im.: immunotherapy.
[c] Anti EGFR.
[d] Immunotherapy.
[e] Chemotherapy.
[f] Anti VEGF.

2. Material and methods

2.1. Chemicals and nanoparticle thin film preparation

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$, 99%), tetraoctylammonium bromide (TOA^+Br^-, 99%), decanethiol (DT, 96%), sodium borohydride ($NaBH_4$, 99%), 11-mercaptoundecanoic acid (MUA), 16-Mercaptohexadecanoic acid (MHA, 90%), 3-mercaptopropanoic acid (MPA, 99%) and alkyl dithiols (ADT, HS-$(CH_2)_n$-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, $Au_{2\,nm}$) encapsulated with DT monolayer shells were synthesized by two-phase reduction of $AuCl_4^-$ 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, $Au_{5\,nm}$) were synthesized by a thermally-activated processing route [27,28]. Briefly, a measured amount of the as-