REVIEW

Advances in the Early Detection of Lung Cancer using Analysis of Volatile Organic Compounds: From Imaging to Sensors

Wang Li[1], Hong-Ying Liu[1]*, Zi-Ru Jia[1], Pan-Pan Qiao[1], Xi-Tian Pi[1,2]*, Jun Chen[1], Lin-Hong Deng[1,3]

Abstract

According to the World Health Organization (WHO), 1.37 million people died of lung cancer all around the world in 2008, occupying the first place in all cancer-related deaths. However, this number might be decreased if patients were detected earlier and treated appropriately. Unfortunately, traditional imaging techniques are not sufficiently satisfactory for early detection of lung cancer because of limitations. As one alternative, breath volatile organic compounds (VOCs) may reflect the biochemical status of the body and provide clues to some diseases including lung cancer at early stage. Early detection of lung cancer based on breath analysis is becoming more and more valued because it is non-invasive, sensitive, inexpensive and simple. In this review article, we analyze the limitations of traditional imaging techniques in the early detection of lung cancer, illustrate possible mechanisms of the production of VOCs in cancerous cells, present evidence that supports the detection of such disease using breath analysis, and summarize the advances in the study of E-noses based on gas sensitive sensors. In conclusion, the analysis of breath VOCs is a better choice for the early detection of lung cancer compared to imaging techniques. We recommend a more comprehensive technique that integrates the analysis of VOCs and non-VOCs in breath. In addition, VOCs in urine may also be a trend in research on the early detection of lung cancer.

Keywords: Lung cancer - volatile organic compounds - early detection - electronic nose - sensor

Asian Pac J Cancer Prev, **15 (11)**, 4377-4384

Introduction

Cancer is a leading cause of death in the world. Lung cancer is the most common type among all cancers (Ferlay et al., 2010). Since early detection of lung cancer is not easy and effective therapy for lung cancer at advanced stage (McWilliams et al., 2003; Boedeker et al., 2012) is still lacking, the 5-year survival rate for non-small cell lung cancer that accounts for 85% of all lung cancers is only 17% (Wang et al., 2013). The 5-year survival rate for patients with stage I lung cancer reaches about 75% after receive surgical resections (Tanner et al., 2012). Therefore, early detection of lung cancer is very important, especially for the high-risk individuals such as smokers, individuals with family cancer history, etc.

Many traditional techniques can be used in the early detection of lung cancer, including chest X-ray (CXR), computerized tomography (CT), positron emission tomography (PET), cytology sputum, serum test, and urine test, etc. The detection levels and biomarkers of these techniques are different (vv 1). However, these traditional detection tools have limitations in the early detection of lung cancer.

In the past decades, CXR test showed no significant effect on decreasing the mortality of lung cancer (Brett, 1968; Flehinger et al., 1984; Fontana et al., 1984; Frost et al., 1984; Melamed et al., 1984; Fontana et al., 1986; Kubik and Polak, 1986; Tockman et al., 1997; Marcus et al., 2000; Doria-Rose and Marcus, 2009; Doria-Rose et al., 2009; Oken et al., 2011). The sensitivity of CXR is low, and may bring about high false negative values (Fossella et al., 2003). In addition, the radiation risk of CXR greatly limits its application in the field. Most recently, Mazzone et al. suggested that computer-aided detection on chest radiography may improve the sensitivity of traditional CXR, but further assessment is needed (Mazzone et al., 2013).

CT can provide detailed information about the size and location of the neoplasm (De Wever et al., 2007). However, it still has high false positive values, and may

[1]Key Laboratory of Biorheology Science and Technology, Ministry of Education, College of Bioengineering, [2]Key Laboratories for National Defense Science and Technology of Innovative Micro-nano Devices and System Technology, Chongqing University, Chongqing, [3]Institute of Biomedical Engineering and Health Sciences, Changzhou University, Changzhou, Jiangsu Province, China
**For correspondence: liuhongyingcqu@163.com*

Table 1. Current Techniques for the Early Detection of Lung Cancer

Detection tool / procedure	Detection level	Markers
CXR	Tissular level	Neoplastic tissue
CT	Tissular level	Neoplastic tissue
PET	Tissular level	Neoplastic tissue
Sputum test	Cellular or molecular level	Abnormal cells and methylated gene promoters
Serum test	Cellular or molecular level	Circulating tumor cells, circulating DNA, plasma proteins, telomerase, etc
Urine test	Molecular level	Urine volatile odorants
Breath analysis	Molecular level	Volatile and non-volatile organic compounds

require a bronchoscope guided technique to confirm the existence of cancerous tissues. In addition, CT may cause some complications or even death (Rami Porta, 1999; Hujala et al., 2001; Detterbeck et al.,2003; Holty et al., 2005). According to the National Lung Screening Trial, low dose CT had a reduction of 20% in lung cancer-specific mortality compared to CXR (Aberle et al., 2011). Recently, CT was recommended by the National Comprehensive Cancer Network for the screening of lung cancer in high risk groups. However, little evidence supported this recommendation, and lots of problems still exist in the application of this technique (Field et al., 2012; Spiro and Navani., 2012; Tanner et al., 2012; Xiang et al., 2013). Similarly, PET technique also has limitations in detecting and staging lung cancer, including low spatial resolution, and inconspicuous contrast among different tissues, etc (De Wever et al., 2007). Although combined PET/CT overcame some disadvantages of single CT or PET, but false positive values were still produced in the early detection of lung cancer (Hochhegger and Marchiori EIrion, 2013). Moreover, it is reported that the PET can only detect tumor tissues with at least 110 billion cancerous cells (Karki et al., 2013). In addition, both CT and PET (including combined CT/PET) may deliver radiation to subjects (Semelka et al., 2007). Importantly, relatively high cost of CT and PET limits their application in the early detection of lung cancer.

After reviewing studies about sputum test for lung cancer patients, Ghosal et al. concluded that traditional sputum cytology combined with CXR could not make CXRs alone better in decreasing the mortality of lung cancer (Ghosal et al., 2009). However, it was suggested that gene promoter methylation in sputum had potential in the early detection of lung cancer (Belinsky et al., 2005; Leng et al., 2012), but more evaluation was needed. In addition, VOCs in urine showed high sensitivity and specificity in the early detection of lung cancer, but still needs larger number of study samples (Hanai et al., 2012).

More and more studies are using biomarkers in serum to detect lung cancer (Tanaka et al., 2002; Sozzi et al., 2003; Miyazu et al., 2005). For example, Yang et al. reported that 5 proteins in serum were successfully used to detect non-small cell lung cancer with a sensitivity of 87% and specificity of 80% (Yang et al., 2005). Circulating miRNAs may have promising future applications for screening and early detection of lung cancer (Ramshankar V and Krishnamurthy A, 2013; Yao et al., 2014). Nevertheless, the sensitivity and specificity of this technique is not high enough for the early detection of lung cancer in large groups. Besides, this technique is invasive, and may make subjects feel unacceptable.

Recently, breath VOCs were used as bio-markers to identify cancers, especially to detect lung cancer (Phillips et al., 1999; McCulloch et al., 2006; Peng et al., 2010). It is suggested that breath analysis may be a good choice for the early detection of lung cancer because it is non-invasive, sensitive, simple and potentially cheaper compared to traditional techniques (Hakim et al., 2012). These desired characteristics are now encouraging many researchers to study the application of this technique.

Mechanism of Breath VOCs Production

Breath VOCs are usually collected from human breath. More than 3000 different VOCs have been identified in human breath (Phillips et al., 1999) since Pauling et al. found almost 250 kinds of breath VOCs in 1971 (Pauling et al., 1971). Concentrations of these VOCs range from parts per million by volume (ppmv) to parts per trillion by volume (pptv). However, the production mechanism of some VOCs can be rationally explained in clinic but that of other VOCs has not been understood clearly until now (Hakim et al., 2012).

The hypothesis of oxidative stress was used to explain the carcinogenesis and the mechanism of breath VOCs (Kneepkens et al., 1994; Marnett, 2000). Reactive oxygen species including superoxide (O2-), hydrogen peroxide (H2O2) and hydroxyl radical (·OH) are mostly secreted from mitochondria as byproducts in the process of aerobic metabolism of cells (Poyton et al., 2009). Reactive oxygen species have both beneficial effects at low concentrations and adverse effects at high concentrations (Poli et al., 2004). Typically, these harmful effects can be balanced by some antioxidants (such as superoxide dismutase and catalase) (Halliwell, 1996). Nevertheless, if the concentrations of reactive oxygen species increase, part of them could induce damages to membrane and DNA of cells, cause protein oxidation and lipid peroxidation, and finally lead to the production of VOCs and non-VOCs that are secreted into body liquid and breath (Aksenov et al., 2012). The final result of this process may be cancer, inflammation, cellular senescence, and apoptosis, etc (Valko et al., 2006). Inflammation, irradiation and pollutants in the atmosphere can also contribute to the production of VOCs (Valko et al., 2006). For example, some substances such as polycyclic aromatic hydrocarbons in tobacco smoke may induce the expression of cytochrome P450 (Roos et al., 2004) which will in turn catalyze these substances into VOCs and other compounds (Hakim et al., 2012).

In the early stage of cancer, cancerous cells proliferate quickly. As a result, AMP-activated protein kinase is

usually activated to accelerate the breakdown of lipids, which provides enough ATP to meet the energy requirement of cell proliferation and varies the composition of breath VOCs (Hardie et al., 2006; Mazzone, 2008). In addition, infectious agents, immune cells (Hakim et al., 2012) and even the route of exhalation such as mouth-exhaled breath and nose-exhaled breath (Pysanenko et al., 2008) may also influence the concentrations of breath VOCs. Moreover, it was found that bacteria in the gut could also produce VOCs (Mazzone, 2008; Tisch et al., 2012).

Evidences for the Early Detection of Lung Cancer Based on Breath Analysis

According to the hypothesis of oxidative stress, breath VOCs might reflect biochemical status of the body, which may provide clues to some diseases including lung cancer in the early stage. Recent experimental results also suggested that the early detection of lung cancer based on breath VOC analysis could be reliable and practicable.

Canine scent detection of lung cancer

Lots of experiments have been performed since Williams and Pembroke detected malignant melanoma using sniffer dogs (Williams and Pembroke, 1989). In 2006, McCulloch et al. recruited 55 lung cancer patients, 31 breast cancer patients and 83 healthy subjects as controls. Five dogs were trained to sniff the breath samples of all the subjects. In double-blind conditions, this experimental result showed 99% sensitivity and 99% specificity in the correct identification of lung cancer patients and controls, which were similar to those observed across all 4 stages of the disease (McCulloch et al., 2006). In 2012, Ehmann et al. reported that their study yielded high specificity (93%) and moderate sensitivity (71%) in the detection of lung cancer from healthy controls using specially trained dogs to detect breath samples (Ehmann et al., 2012). Although the specific constituents in human breath working in canine scent detection of lung cancer are still unknown, preliminary success with canine scent detection suggests that breath analysis is practicable in the early detection of lung cancer (Moser and McCulloch, 2010).

Lung cancer detection using spectroscopic techniques and sensors

In recent decades, researchers studied VOCs in human breath using spectroscopic techniques and found that breath VOCs from lung cancer patients and healthy controls were different. Gordon et al. analyzed 3 VOCs (acetone, methylethylketone and n-propanol) in human breath using gas chromatography-mass spectrometry to identify lung cancer patients from 29 subjects and obtained an accuracy of 93% (Gordon et al., 1985). In 1999, Phillips et al. collected 108 breath samples (60 from lung cancer patients and 48 from controls) and used 22 VOCs as markers to identify patients with lung cancer, with a sensitivity of 100% and a specificity of 81.3% after being quantified and identified by mass spectroscopy (Phillips et al., 1999). In 2003, this research group used 9 VOCs as markers to analyze breath samples from 108 subjects (67 primary lung cancer patients and 41 controls) with a sensitivity of 89.6% and a specificity of 82.9% (Phillips et al., 2003). In 2007, on 193 patients with primary lung cancer and 211 healthy controls, this research group analyzed 16 VOCs by gas chromatography and mass spectrometry, employed a fuzzy logic analysis as the analysis model, and acquired a sensitivity of 84.6% and specificity of 80% (Phillips et al., 2007). In 2010, Peng et al. reported that a designed nano-sensor array could distinguish lung cancer from other cancers after analyzing breath samples of 96 subjects (30 with primary lung cancer, 26 with primary colon cancer, 18 with primary prostate cancer and 22 healthy controls) (Peng et al., 2010). All these encouraging results indicate that the discrimination of lung cancer from other cancers and controls can be achieved by using modern technology although some experimental sensitivities and specificities are not high enough for the early detection of lung cancer.

Studies on VOCs in headspace of lung cancer cell lines

After investigating the production of VOCs from cancer cell lines (SK-MES and CALU-1) in vitro, Smith et al. found that the concentration of acetaldehyde in the headspace of the medium/cell culture was proportional to the number of cancer cells (Smith et al., 2003). Most recently, Peled et al. reported a study on VOCs in the headspace of lung cancer cells with different genetic mutations, in which 5 VOCs were found to be related to specific mutations of lung cancer cells (Peled et al., 2013).

Other results were also observed in molecular biology studies of lung cancer cells. It is reported that the expression levels of superoxide dismutase and catalase are different between lung cancer cells and normal cells, and this may affect the oxidation of lipids and vary the final composition of VOCs (Chung-man Ho et al., 2001). Other reports claimed that the mutation of liver kinase B1 (LKB1), an upstream kinase of the AMP-activated protein kinase, was found in lung cancer cells (Zhong et al., 2006). The mutation of LKB1 could change the expression level of AMP-activated protein kinase, and finally influence the metabolism of lipids and the production of VOCs.

Current Techniques for the Analysis of Breath VOCs

Traditional techniques

In the past decades, lots of spectroscopic techniques including mass spectrometry-based techniques and laser absorption spectroscopy-based techniques were used to analyze breath samples. Mass spectrometry-based techniques include gas chromatography-mass spectrometry, selected ion flow tube-mass spectrometry, and proton transfer reaction-mass spectrometry, etc. Laser absorption spectroscopy-based techniques mainly include cavity ring down spectroscopy, tunable diode laser absorption spectroscopy and photo-acoustic spectroscopy. Some other spectroscopic techniques were also employed to analyze breath samples, such as ion mobility spectrometry and Raman scattering. A comparison of all these techniques used for breath analysis was summarized by Chow and colleagues. The techniques based on

mass spectrometry are always reliable and sensitive but complicated and expensive in analyzing breath samples. A pre-concentration process is usually needed to improve the sensitivities of these techniques and an expert is required to operate the equipment. Notably, molecules in breath samples with the same m/z ratios could produce overlaps in signals due to the working mechanism of mass spectrometry, making them difficult to be distinguished (Chow et al., 2012). Laser absorption spectroscopy-based techniques are simple, cheap and relatively quicker than mass spectrometry-based techniques, but not easy to simultaneously detect so many VOCs in breath due to the limitation of the spectral range (Chow et al., 2012). Because of the limitations of spectroscopic techniques, a simple, cost-effective and reliable analysis technique is desired for clinical use.

Electronic noses

Enlightened by olfactory system in mammals and encouraged by the experimental results of canine scent detection of lung cancer, a series of electronic noses (E-noses) were developed by researchers to analyze breath VOCs. This opened a new prospect for the early detection of lung cancer. Herein, the advances of several E-noses were reported in the detection of lung cancer in recent years are reviewed.

E-nose based on quartz crystal microbalance sensors

A quartz crystal microbalance (QCM) sensor mainly consists of gold electrodes, sensitive coating and quartz wafer (Figure 1). Gas sensitive materials such as metalloporphyrins are always used as sensitive coatings in gas sensitive QCM sensors. When the QCM sensor is exposed to breath sample, molecules of VOCs in the breath sample could be adsorbed on the sensitive coating, which causes mass change (Δm) on the surface of the sensor. And then, the oscillation frequency change of the quartz wafer (Δf) is induced because of piezoelectricity of this thin quartz crystal, which can be formulated by a relation known as Sauerbrey law as follows (Sauerbrey, 1959).

$$\triangle f=(-2f_0^2\triangle m)\div[A(\mu_q\rho_q)]^{1/2} \quad (1)$$

Where Δf is the frequency shift, f_o is the fundamental frequency, Δm is the mass change, A is the effective area, μ_q is the shear modulus ($2.95\times1{,}010$ Kg·m^{-1}·s^{-2}), and ρ_q is the density of quartz (2,648 Kg·m^{-3}). VOCs with different compositions have different total mass which can be measured by counting the oscillation frequency of quartz wafer. Therefore, lung cancer can be detected based on the "breath pattern" demonstrated by oscillation frequency. In 2003, Di Natale et al. (2003) employed an E-nose comprising 8 QCM gas sensors coated with different metalloporphyrins to analyze breath samples of 35 lung cancer patients and 18 controls (Di Natale et al., 2003). The sensors showed good sensitivity (100%) and specificity (94%) in distinguishing lung cancer patients from controls when "partial least squares-discriminant analysis" was used. In 2010, the same E-nose was used to analyze breath samples from 28 patients with lung cancer, 28 patients with other lung diseases (16 with chronic obstructive pulmonary diseases, 5 with bronchitis, 4 with pleurisy and 3 with interstitial lung disease) and 36 healthy controls. Good discrimination was achieved: the sensitivity and specificity for lung cancer patients and controls were 85% and 100%, respectively; the sensitivity and specificity for patients with lung cancer and patients with other lung disease were 92.8% and 78.6%, respectively; the sensitivity and specificity for lung cancer patients and controls were 89.3% and 79.3%, respectively (D'Amico et al., 2010). In 2012, the same E-nose was also used to analyze the breath samples of 30 subjects (10 with adenocarcinoma, 10 with squamous cell carcinoma and 10 controls) using two breath collection strategies (bag breath sampling and endoscopic breath sampling). Overall 90% and 85% correct classifications were observed by using endoscopic breath sampling and bag breath sampling, respectively (Santonico et al., 2012).

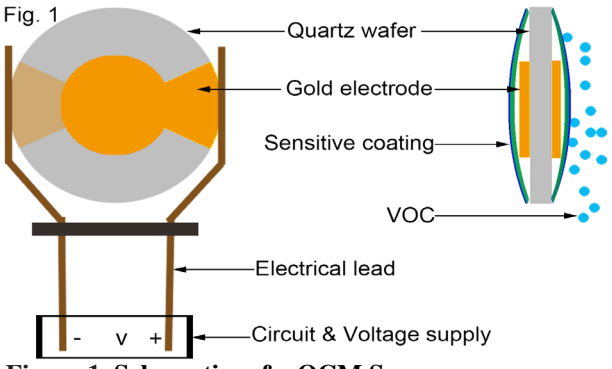

Figure 1. Schematics of a QCM Sensor

E-nose based on surface acoustic wave sensors

With a similar principle but greater sensitivity compared to QCM sensors, surface acoustic wave (SAW) sensors can also be used to analyze breath VOCs adsorbed by sensitive film coated on the surface of the sensors. Illustration on how SAW sensor works is shown in Figure 2. An oscillating voltage or electric field upon the piezoelectric substrate can generate acoustic waves which propagate on the surface of the sensors because of the piezoelectricity of the substrate. When exposed to breath samples, gas sensitive layer coated on the surface of the substrate adsorbs some specific VOCs, and then causes mass changes of the layer and velocity changes of the SAW, which can be monitored by counting the frequency of the sensor. An equation between changes of the coating mass and the oscillation frequency was proposed by Auld and developed by Wohltjen (1984). The equation can be simply formulated as follows:

$$\triangle f=(k_1+k_2)\, f_0^2\, h\rho \quad (2)$$

Where Δf is the frequency shift of the sensor, $k1$ and $k2$ are material parameters of the substrate, fo is the fundamental frequency of the sensor, and h and ϱ are the thickness and density of the layer, respectively. The curve of frequency change is always used as "breath pattern" to identify lung cancer.

This kind of E-nose was reported to detect lung cancer patients with satisfactory results (Yu et al., 2003). In 2005, Chen et al. also reported that the E-nose based on SAW sensors was effective in identifying lung cancer patients while using 11 VOCs as biomarkers(Chen et al., 2005). In 2011, a detection system combining SAW devices with metal oxide semiconductor units was proposed by

Fig. 2

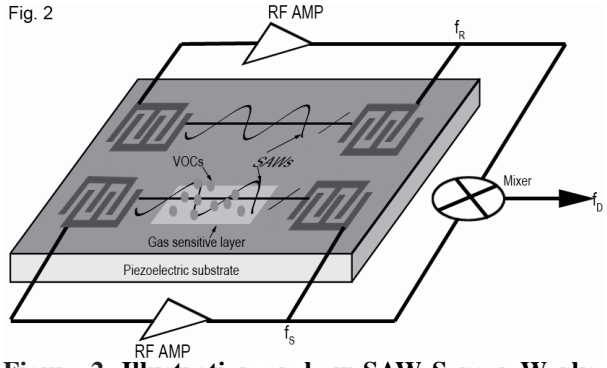

Figure 2. Illustration on how SAW Sensor Works. A second SAW device is used as a reference to minimize the temperature drift (fS is sample frequency, fR is reference frequency and fD is frequency difference)

a research group in Zhejiang University (Wang et al., 2012). After testing 89 breath samples, an artificial neural network model was established to analyze the testing result, and high sensitivity of 93.62% and selectivity of 83.37% were obtained, suggesting that this system was promising in the early detection of lung cancer.

E-nose based on gold nanoparticle sensors

An E-nose based on gold nanoparticle (GNP) sensors was designed to detect lung cancer by a research group in Israel Institute of Technology (Barash et al., 2009). This E-nose is composed of 18 cross-reactive chemiresistors that are mounted into a polytetrafluoroethylene circuit board. Each resistor consists of silicon wafer, thermal oxide (300 nm in thickness), circular interdigitated gold electrodes and chemiresistive layer. The chemiresistive layer is formed by drop-casting the solution of gold nanoparticles coated with organic ligands onto the gold electrodes. Organic ligands could provide wide adsorption sites for VOCs in breath, and different ligands may adsorb different VOCs. When exposed to breath samples, all sensors varies in conductivity with the adsorption of different VOCs, and finally the varying pattern of resistivity of all 18 sensors can be used to identify lung cancer patients.

By using this technique, Haick and colleagues analyzed gas samples in human breath and headspace of lung cancer cell lines in vitro (Peng et al., 2009; Peng et al., 2010). In 2012, Barash et al. analyzed VOCs in the headspace of 10 adenocarcinoma cell lines, 4 squamous cell carcinoma cell lines, 4 small cell lung cancer cell lines and 1 immortal bronchial epithelium cell line using GNP sensors. By employing support vector machine algorithm, a significantly discriminative power of this E-nose was observed (lung cancer *vs* immortal bronchial epithelium: 96% sensitivity and 86% specificity; non-small cell lung cancer *vs* small cell lung cancer: 100% sensitivity and 75% specificity; adenocarcinoma *vs* squamous cell carcinoma: 86% sensitivity and 100% specificity) (Barash et al., 2012). In 2013, Peled et al. reported that 5 VOCs in the headspace of lung cancer cell lines with 3 different genetic mutations and corresponding wild-type cell lines are different from each other. At last, a good classification (with 84%-96% accuracy) among them was reached by using GNP sensors (Peled et al., 2013).

E-nose based on colorimetric sensors

Colorimetric sensors are a kind of optical sensors. Recently, a colorimetric sensor containing arrays of chemically responsive pigments was designed to differentiate different types of VOCs (Janzen et al., 2006; Muro et al., 2008; Hou et al., 2013). Dyes such as metalloporphyrins, basic/acid indicators, and redox dyes are printed inside a disposable cartridge. When the sensor is exposed to gas vapor, each spot changes its color according to the chemical environment. Then, a digital camera or a scanner is used to monitor the color change of the cartridge before and after exposure to the analytes. Finally, the color difference maps are analyzed by computer to identify different VOCs.

In 2007, Mazzone et al. used a colorimetric sensor with 36 dye spots and a random forest model to predict the presence of lung cancer, and the detection of lung cancer has 73.3% sensitivity and 72.4% specificity (Mazzone et al. 2007). In 2012, Mazzone and colleagues analyzed breath samples of 229 subjects (92 lung cancer patients and 137 controls) by employing an improved colorimetric sensor with 24 dye spots. A moderate accuracy (C-statistic 0.811) in distinguishing lung cancer patients from controls and a better accuracy (C-statistic 0.899) in distinguishing adenocarcinoma from squamous cell carcinoma were observed, which was comparable with low dose CT (Mazzone et al., 2012).

In 2011, a research group in Chongqing University developed an artificial tongue system based on colorimetric sensor array using chemical responsive dyes, porphyrins and their derivatives as sensing elements (Hou et al., 2011), and the sensor showed an excellent capability in the correct identification and quantificational analysis of protein samples. In 2013, the same sensor was employed to distinguish lung cancer related VOCs, and correctly identified different concentrations of all 4 VOCs (Hou et al., 2013).

E-nose based on conductive polymer composite (CPC) sensors

The CPC sensors are generally constructed by depositing solutions containing conductive filler and insulating matrix onto interdigitated electrodes (Albert et al., 2000). Generally, carbon black and polypyrrole are always used as the conductive materials in CPC sensors. To increase the diversity of the sensor array, different kinds of organic polymers are used as insulating matrices. When the sensor is exposed to VOCs, gaseous analytes can interact with both conductive filler and insulating matrix, and then, the composite will swell to change the conductivity of the coating film (Albert et al., 2000). Sensors containing different insulating matrices may respond differently to a given odorant. Finally, a distinctive pattern can be obtained to distinguish various VOCs. The resistivity of the composite can be predicted on the basis of percolation theory according to an equation as follows (Brosseau et al., 1997).

$$\rho=[(z-2)\rho_c\rho_m] \div \{A+B+[(A+B)^2+2(z-2)\rho_c\rho_m]^{1/2}\} \quad (3)$$

where

$$A=\rho_c\{-1+(z\div 2)[1-(v_c\div f)]\} \quad (4)$$

$$B=\rho_m[(zv_c \div 2f)-1] \quad (5)$$

Where ρ is the resistivity of the conductive composite, ϱc is the resistivity of the conductive filler, ϱm is the resistivity of the insulating matrix, z is the coordination number of the conductive filler particles, vc is the volume fraction of the conductive filler in the composite, and f is their total packing fraction ($vc < f$).

In 2011, Castro and co-workers optimized CPC sensors by varying the amount of carbon nanotubes as conductive filler with 5 different polymer matrices (Castro et al., 2011). Finally, high sensitivity and selectivity, as well as good rapidity and reproducibility, were obtained when the sensor array was exposed to 9 VOCs that were chosen among biomarkers for lung cancer detection. This result may make the CPC sensors practical in the early detection of lung cancer.

Although encouraging results have been achieved in the detection of lung cancer by using E-noses, there are still challenges in the application of this technique (Wilson and DBaietto, 2009). E-noses are sometimes vulnerable to interference (humidity, temperature, etc.) that may produce noise in breath patterns. The sensitivity of some sensors is low, and a pre-concentration procedure is always needed. In addition, some sensors have a relatively short life and none of the E-noses can provide quantitative data of single VOC.

Conclusions

Lung cancer is the leading cause of cancer deaths in the world, but early detection of lung cancer may greatly increase the survival rate of the patients. Traditional techniques are not always satisfactory in the early detection of lung cancer due to their various limitations. Analysis of breath VOCs may be a good choice for the early detection of lung cancer, because it is non-invasive, sensitive, simple and potentially more inexpensive. Moreover, recent experimental results suggested that the analysis of breath VOCs is reliable and practicable in the early detection of lung cancer. Spectroscopic techniques did not seem applicable for analyzing breath VOCs in clinical practice, but E-noses showed promising prospects.

As a developing technique, breath analysis for the early detection of lung cancer is not faultless or at least not good enough for clinical use at present. All of the related experiments are based on a small number of patients, and disputes still widely exist on whether breath analysis could detect early lung cancer. Traditional gas analysis techniques are too sophisticated and expensive for clinical use, and E-noses are now facing challenges in solving their own limitations.

Therefore, prior considerations for future studies should include: i) the finalization of the markers in breath VOCs; ii) the standardization of the methods and procedures for the analysis of breath VOCs; iii) the decrease of interferences and the improvement in sensitivity and stability for the demands in clinical use. At last, we propose a more comprehensive technique that integrates the analysis of VOCs and non-VOCs in breath. In addition, VOCs in urine may also be a trend in the research on the early detection of lung cancer.

Acknowledgements

This work was supported by the National Nature Science Foundation of China (No. 81101172), the National Key Technologies R&D Program (No. 2012BAI19B03 and 2011BAI08B12) and the Scientific Research Foundation for Returned Scholars of China.

References

Aberle DR, Adams AM, Berg CD, et al (2011). Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med*, **365**, 395-409.

Aksenov AA, Gojova A, Zhao W, et al (2012). Characterization of volatile organic compounds in human leukocyte antigen heterologous expression systems: a cell's "chemical odor fingerprint". *Chembiochem*, **13**, 1053-9.

Albert KJ, Lewis NS, Schauer CL, et al (2000). Cross-reactive chemical sensor arrays. *Chem Rev*, **100**, 2595-626.

Barash O, Peled N, Hirsch FR, Haick H (2009). Sniffing the unique "odor print" of non small cell lung cancer with gold nanoparticles. *Small*, **5**, 2618-24.

Barash O, Peled N, Tisch U, et al (2012). Classification of lung cancer histology by gold nanoparticle sensors. *Nanomedicine*, **8**, 580-9.

Belinsky SA, Klinge DM, Dekker JD, et al (2005). Gene promoter methylation in plasma and sputum increases with lung cancer risk. *Clin Cancer Res*, **11**, 6505-11.

Boedeker E, Friedel G, Walles T (2012). Sniffer dogs as part of a bimodal bionic research approach to develop a lung cancer screening. *Interact Cardiovasc Thorac Surg*, **14**, 511-5.

Brett GZ (1968). The value of lung cancer detection by six-monthly chest radiographs. *Thorax*, **23**, 414-20.

Brosseau C, Boulic F, Queffelec P (1997). Dielectric and microstructure properties of polymer carbon black composites. *J Appl Phys*, **81**, 882-91.

Castro M, Kumar B, Feller JF (2011). Novel e-nose for the discrimination of volatile organic biomarkers with an array of carbon nanotubes (CNT) conductive polymer nanocomposites (CPC) sensors. *Sens Actuators B: Chemical*, **159**, 213-9.

Chen X, Cao MF, Li Y (2005). A study of an electronic nose for detection of lung cancer based on a virtual SAW gas sensors array and imaging recognition method. *Meas Sci Technol*, **16**, 1535-46.

Chow KK, Short M, Zeng H (2012). A comparison of spectroscopic techniques for human breath analysis. *Biomedical Spectroscopy Imaging*, **1**, 339-53.

Chung-man Ho J, Zheng S, Comhair SA, et al (2001). Differential expression of manganese superoxide dismutase and catalase in lung cancer. *Cancer Res*, **61**, 8578-85.

D'Amico A, Pennazza G, Santonico M, et al (2010). An investigation on electronic nose diagnosis of lung cancer. *Lung Cancer*, **68**, 170-6.

De Wever W, Ceyssens S, Mortelmans L, et al (2007). Additional value of PET-CT in the staging of lung cancer: comparison with CT alone, PET alone and visual correlation of PET and CT. *Eur Radiol*, **17**, 23-32.

Detterbeck FC, DeCamp MM Jr, Kohman LJ, Silvestri GA (2003). Lung cancer. Invasive staging: the guidelines. *Chest*, **123**, 167-75.

Di Natale C, Macagnano A, Martinelli E, et al (2003). Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. *Biosens Bioelectron*, **18**, 1209-18.

Doria-Rose VP, Marcus PM (2009). Death certificates provide an adequate source of cause of death information when

evaluating lung cancer mortality: An example from the Mayo Lung Project. *Lung Cancer*, **63**, 295-300.

Doria-Rose VP, Marcus PM, Szabo E, et al (2009). Randomized controlled trials of the efficacy of lung cancer screening by sputum cytology revisited a combined mortality analysis from the Johns Hopkins Lung Project and the Memorial Sloan-Kettering Lung Study. *Cancer*, **115**, 5007-17.

Ehmann R, Boedeker E, Friedrich U, et al (2012). Canine scent detection in the diagnosis of lung cancer: revisiting a puzzling phenomenon. *Eur Respir J*, **39**, 669-76.

Ferlay J, Shin HR, Bray F, et al (2010). GLOBOCAN 2008, Cancer incidence and mortality worldwide: IARC CancerBase No. 10. 2010 [Internet]: Lyon, France: International Agency for Research on Cancer.

Field JK, Smith RA, Aberle DR, et al (2012). International Association for the Study of Lung Cancer Computed Tomography Screening Workshop 2011 report. *J Thorac Oncol*, **7**, 10-9.

Flehinger BJ, Melamed MR, Zaman MB, et al (1984). Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Memorial Sloan-Kettering study. *Am Rev Respir Dis*, **130**, 555-60.

Fontana RS, Sanderson DR, Taylor WF, et al (1984). Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Mayo Clinic study. *Am Rev Respir Dis*, **130**, 561-5.

Fontana RS, Sanderson DR, Woolner LB, et al (1986). Lung-Cancer Screening - the Mayo Program. *J Occup Med*, **28**, 746-50.

Fossella F, Komaki R, Putnam J (2003). Lung Cancer. MD Anderson Cancer Care Series. New York, NY Springer-Verlag.

Frost JK, Ball WC Jr, Levin ML, et al (1984). Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Johns Hopkins study. *Am Rev Respir Dis*, **130**, 549-54.

Ghosal R, Kloer P, Lewis KE (2009). A review of novel biological tools used in screening for the early detection of lung cancer. *Postgrad Med J*, **85**, 358-63.

Gordon SM, Szidon JP, Krotoszynski BK, et al (1985). Volatile organic compounds in exhaled air from patients with lung cancer. *Clin Chem*, **31**, 1278-82.

Hakim M, Broza YY, Barash O, et al (2012). Volatile organic compounds of lung cancer and possible biochemical pathways. *Chem Rev*, **112**, 5949-66.

Halliwell B (1996). Antioxidants in human health and disease. *Annu Rev Nutr*, **16**, 33-50.

Hanai Y, Shimono K, Matsumura K, et al (2012). Urinary volatile compounds as biomarkers for lung cancer. *Biosci Biotechnol Biochem*, **76**, 679-84.

Hardie DG, Hawley SA, Scott JW (2006). AMP-activated protein kinase--development of the energy sensor concept. *J Physiol*, **574**, 7-15.

Hochhegger B, Marchiori EIrion K (2013). MRI in lymph node staging of lung cancer. *AJR Am J Roentgenol*, **200**, 540.

Holty JE, Kuschner WG, Gould MK (2005). Accuracy of transbronchial needle aspiration for mediastinal staging of non-small cell lung cancer: a meta-analysis. *Thorax*, **60**, 949-55.

Hou C, Dong J, Zhang G, et al (2011). Colorimetric artificial tongue for protein identification. *Biosens Bioelectron*, **26**, 3981-6.

Hou C, Lei J, Huo D (2013). Discrimination of Lung Cancer Related Volatile Organic Compounds with a Colorimetric Sensor Array. *Anal Lett*, **46**, 2048-59.

Hujala KT, Sipila JI, Grenman R (2001). Mediastinoscopy--its role and value today in the differential diagnosis of mediastinal pathology. *Acta Oncol*, **40**, 79-82.

Janzen MC, Ponder JB, Bailey DP, et al (2006). Colorimetric sensor arrays for volatile organic compounds. *Anal Chem*, **78**, 3591-600.

Karki S, Yin Yj, Samanai N et al (2013). Breathe analyzer and its importance for the early detection of lung cancer. *Sky J Med Med Sci*, **1**, 7-9.

Kneepkens CM, Lepage G, Roy CC (1994). The potential of the hydrocarbon breath test as a measure of lipid-peroxidation. *Free Radic Biol Med*, **17**, 127-60.

Kubik A, Polak J (1986). Lung cancer detection. Results of a randomized prospective study in Czechoslovakia. *Cancer*, **57**, 2427-37.

Leng S, Do K, Yingling CM, et al (2012). Defining a gene promoter methylation signature in sputum for lung cancer risk assessment. *Clin Cancer Res*, **18**, 3387-95.

Marcus PM, Bergstralh EJ, Fagerstrom RM, et al (2000). Lung cancer mortality in the Mayo Lung Project: impact of extended follow-up. *J Natl Cancer Inst*, **92**, 1308-16.

Marnett LJ (2000). Oxyradicals and DNA damage. *Carcinogenesis*, **21**, 361-70.

Mazzone PJ (2008). Analysis of volatile organic compounds in the exhaled breath for the diagnosis of lung cancer. *J Thorac Oncol*, **3**, 774-80.

Mazzone PJ, Hammel J, Dweik R, et al (2007). Diagnosis of lung cancer by the analysis of exhaled breath with a colorimetric sensor array. *Thorax*, **62**, 565-8.

Mazzone PJ, Obuchowski N, Phillips M, et al (2013). Lung cancer screening with computer aided detection chest radiography: design and results of a randomized, controlled trial. *PLOS ONE*, **8**, 59650.

Mazzone PJ, Wang XF, Xu Y, et al (2012). Exhaled breath analysis with a colorimetric sensor array for the identification and characterization of lung cancer. *J Thorac Oncol*, **7**, 137-42.

McCulloch M, Jezierski T, Broffman M, et al (2006). Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. *Integr Cancer Ther*, **5**, 30-9.

McWilliams A, Mayo J, MacDonald S, et al (2003). Lung Cancer Screening A Different Paradigm. *Am J Respir Crit Care Med*, **168**, 1167-73.

Melamed MR, Flehinger BJ, Zaman MB, et al (1984). Screening for early lung cancer. Results of the Memorial Sloan-Kettering study in New York. *Chest*, **86**, 44-53.

Miyazu YM, Miyazawa T, Hiyama K, et al (2005). Telomerase expression in noncancerous bronchial epithelia is a possible marker of early development of lung cancer. *Cancer Res*, **65**, 9623-7.

Moser E, McCulloch M (2010). Canine scent detection of human cancers: A review of methods and accuracy. *J Vet Behav*, **5**, 145-52.

Muro ML, Daws CA, Castellano FN (2008). Microarray pattern recognition based on Pt(II) terpyridyl chloride complexes: vapochromic and vapoluminescent response. *Chem Commun*, **14**, 6134-6.

Oken MM, Hocking WG, Kvale PA, et al (2011). Screening by Chest Radiograph and Lung Cancer Mortality The Prostate, Lung, Colorectal, and Ovarian (PLCO) Randomized Trial. *JAMA*, **306**, 1865-73.

Pauling L, Robinson AB, Teranishi R, Cary P (1971). Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. *Proc Natl Acad Sci USA*, **68**, 2374-6.

Peled N, Barash O, Tisch U, et al (2013). Volatile fingerprints of cancer specific genetic mutations. *Nanomed Nanotechnol*, **9**, 758-66.

Peng G, Hakim M, Broza YY, et al (2010). Detection of lung,

breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. *Br J Cancer*, **103**, 542-51.

Peng G, Tisch U, Adams O, et al (2009). Diagnosing lung cancer in exhaled breath using gold nanoparticles. *Nat Nanotechnol*, **4**, 669-73.

Phillips M, Altorki N, Austin JHM (2007). Prediction of lung cancer using volatile biomarkers in breath. *Cancer Biomark*, **3**, 95-109.

Phillips M, Cataneo RN, Cummin AR, et al (2003). Detection of lung cancer with volatile markers in the breath. *Chest*, **123**, 2115-23.

Phillips M, Gleeson K, Hughes JM, et al (1999). Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. *Lancet*, **353**, 1930-3.

Phillips M, Herrera J, Krishnan S, et al (1999). Variation in volatile organic compounds in the breath of normal humans. *J Chromatogr B Biomed Sci Appl*, **729**, 75-88.

Poli G, Leonarduzzi G, Biasi F, Chiarpotto E (2004). Oxidative stress and cell signalling. *Curr Med Chem*, **11**, 1163-82.

Poyton RO, Ball KA, Castello PR (2009). Mitochondrial generation of free radicals and hypoxic signaling. *Trends Endocrinol Metab*, **20**, 332-40.

Pysanenko A, Spaněl P, Smith D (2008). A study of sulfur-containing compounds in mouth- and nose-exhaled breath and in the oral cavity using selected ion flow tube mass spectrometry. *J Breath Res*, **2**, 046004.

Rami Porta R (1999). Surgical exploration of the mediastinum by mediastinoscopy, parasternal mediastinotomy and remediastinoscopy: indications, technique and complications. *Ann Ital Chir*, **70**, 867-72.

Ramshankar V, Krishnamurthy A (2013). Lung cancer detection by screening - presenting circulating miRNAs as a promising next generation biomarker breakthrough. *Asian Pac J Cancer Prev*, **14**, 2167-72.

Roos PH, Tschirbs S, Pfeifer F, et al (2004). Risk potentials for humans of original and remediated PAH-contaminated soils: application of biomarkers of effect. *Toxicology,* **205**, 181-94.

Santonico M, Lucantoni G, Pennazza G, et al (2012). In situ detection Of lung cancer volatile fingerprints using bronchoscopic air-sampling. *Lung Cancer*, **77**, 46-50.

Sauerbrey G (1959). Use of vibrating quartz for thin film weighing and microweighing. *Z Phys*, **155**, 206-22.

Semelka RC, Armao DM, Elias J Jr, Huda W (2007). Imaging strategies to reduce the risk of radiation in CT studies, including selective substitution with MRI. *J Magn Reson Imaging*, **25**, 900-9.

Smith D, Wang T, Sulé-Suso J, et al (2003). Quantification of acetaldehyde released by lung cancer cells in vitro using selected ion flow tube mass spectrometry. *Rapid Commun Mass Spectrom*, **17**, 845-50.

Sozzi G, Conte D, Leon M, et al (2003). Quantification of free circulating DNA as a diagnostic marker in lung cancer. *J Clin Oncol*, **21**, 3902-8.

Spiro SG, Navani N (2012). Screening for lung cancer: Is this the way forward? *Respirology*, **17**, 237-46.

Tanaka K, Akechi T, Okuyama T, et al (2002). Prevalence and screening of dyspnea interfering with daily life activities in ambulatory patients with advanced lung cancer. *J Pain Symptom Manage*, **23**, 484-9.

Tanner NT, Mehta H, Silvestri GA (2012). New testing for lung cancer screening. *Oncology*, **26**, 176-82.

Tisch U, Billan S, Ilouze M (2012). Volatile organic compounds in exhaled breath as biomarkers for the early detection and screening of lung cancer. *CML Lung Cancer*, **5**, 107-17.

Tockman MS, Mulshine JL (1997). Sputum screening by quantitative microscopy: a new dawn for detection of lung cancer? *Mayo Clin Proc*, **72**, 788-90.

Valko M, Rhodes CJ, Moncol J, et al (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact*, **160**, 1-40.

Wang D, Yu K, Wang Y (2012). A hybrid electronic noses'system based on mos-saw detection units intended for lung cancer diagnosis. *J Innov Opt Health Sci*, **5**, 1150006.

Wang Y, Gu J, Roth JA, et al (2013). Pathway-based serum microRNA profiling and survival in patients with advanced non-small cell lung cancer. *Cancer Res*, **73**, 4801-9.

Williams H, Pembroke A (1989). Sniffer dogs in the melanoma clinic? *Lancet*, **1**, 734.

Wilson A D, Baietto M (2009). Applications and advances in electronic-nose technologies. *Sensors (Basel)*, **9**, 5099-148.

Wohltjen H (1984). Mechanism of operation and design considerations for surface acoustic-wave device vapor sensors. *Sens Actuators*, **5**, 307-25.

Xiang D, Zhang B, Doll D, et al (2013). Lung cancer screening: from imaging to biomarker. *Biomarker Res*, **1**, 1-10.

Yang SY, Xiao XY, Zhang WG, et al (2005). Application of serum SELDI proteomic patterns in diagnosis of lung cancer. *BMC Cancer*, **5**, 83.

Yao Q, Sun JG, Ma H, et al (2014). Monitoring microRNAs using a molecular beacon in CD133+/ CD338+ human lung adenocarcinoma-initiating A549 cells. *Asian Pac J Cancer Prev*, **15**, 161-6.

Yu H, Xu L, Cao MF (2003). Detection volatile organic compounds in breath as markers of lung cancer using a novel electronic nose. *Proceedings of the IEEE Sensors*, **2**, 1333-7.

Zhong D, Guo L, de Aguirre I, et al (2006). LKB1 mutation in large cell carcinoma of the lung. *Lung Cancer*, **53**, 285-94.