



A handheld electronic device with the potential to detect lung cancer biomarkers from exhaled breath

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Abstract

Lung cancer is the leading cause of cancer death in the United States. It has the lowest 5-year survival rate among the most common cancers and therefore, early diagnosis is critical to improve the survival rate. In this paper, a new handheld electronic device is proposed to detect nine lung cancer biomarkers in the exhaled breath. An electrochemical gas sensor was produced through deposition of a thin layer of graphene and Prussian blue on a chromium-modified silicon substrate. Selective binding of the analyte was formed by molecular imprinting polymer (MIP). Subsequent polymerization and removal of the analyte yielded a layer of a conductive polymer on top of the sensor containing molecularly imprinted cavities selective for the target molecule. The sensors were tested over 1–20 parts per trillion (ppt) level of concentration while the sensor resistance has been monitored as the sensors react to the analyte by resistance change. Pentane sensor was also tested for selectivity. A printed circuit board was designed to measure the resistance of each sensor and send the data to a developed application in smartphone through Bluetooth. This handheld device has the potential to be used as a diagnostic method in the near future.

Index Terms Bluetooth · Diagnosis · Electrochemical · Gas sensor · Lung cancer

1 Introduction

Cancer is a category of diseases that happens by uncontrolled growth and spread of abnormal cells by the definition of the American Cancer Society. Lung cancer (LC) is the leading cause of cancer mortality. There are two major forms of LC disease: non-small tumor LC (NSCLC; 85% of all LCs) and small tumor LC (SCLC; 15% of all LCs). Symptoms include persistent cough, sputum streaked with blood, chest pain, voice change, worsening shortness of breath, and recurrent pneumonia or bronchitis. However, the symptoms usually do not appear until the advanced stage of the cancer (Viale 2020). Treatment options are based on the size of the tumor and based on that, the patient might go through surgery, chemotherapy, radiation therapy, or targeted drugs and

immunotherapy. The 5-year relative survival rate for lung cancer is 19% and only 16% of lung cancers are diagnosed at an early stage (Viale 2020). Screening with low-dose spiral computed tomography (LDCT) is the only option that has been shown to reduce lung cancer mortality by about 20%. Furthermore, the society recommends annual screening for lung cancer with LDCT in adult smokers 55 to 74 years of age. However, the high false-positive rate (about 97.5%) requires almost all participants to do follow up testing (Kinsinger et al. 2017). A study calculated an average diagnosis assessment cost of almost 9000 patients who were tested with LDCT about \$7567 (Lokhandwala et al. 2017).

Strong correlation of lung cancer survival rate with early diagnosis, high false-positive rate of LDCT, the cost and time of assessment, all prove a high demand for a device that can accurately and instantly detect LC at early stage with minimal cost. We have developed an electronic hand-held device that can be applied toward detecting lung cancer volatile organic compounds (VOCs) in the patient's breath. Impressive preliminary data have confirmed the potential of VOCs to serve as a basis for a noninvasive, simple, cost-effective, and easy-to-use diagnostic tool. In near future, monitoring VOCs may become a substitute to conventional medical

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diagnostics. VOCs of LC can be detected from the headspace of the cancer cells, blood, and/or via exhaled breath (Hakim et al. 2012; Nakhleh et al. 2017; Peng et al. 2009). The Non-volatile products such as lipid hydroperoxides, conjugated dienes and malondialdehydes are not easy to track. On the other hands, the volatile products are hydrocarbons mainly alkanes and methylated alkanes in the exhaled breath (Zieba et al. 2001). Most studies used gas chromatography – mass spectrometry (GC–MS) to identify the LC VOCs (Bajtarevic et al. 2009; Filipiak et al. 2008; Hanna et al. 2019). However, there are other platforms been used in order to identify the VOC biomarkers including Tunable Diode Laser Spectrometer (TDLS) that has been applied to the analysis of exhaled ethane in patients with lung cancer (Skeldon et al. 2006). TDLS is a technique for measuring the concentration of certain species such as methane, water vapor and many more, in a gaseous mixture using tunable diode lasers and laser absorption spectrometry. The working principle is based on a single absorption line in the absorption spectrum of a particular species of interest. The sensitivity of TDLS is at part per billion (ppb) level. Ion mobility spectrometry (IMS) with multi-capillary column (MCC), was also applied to detect VOCs in patients with lung cancer. IMS/ MCC can detect a very low concentration of VOCs normally in the ppb- to ppt-range. However, it will take about 8 min for detection (Handa et al. 2014). Schumer et al. (2016) proposed silicon microchip to detect lung cancer biomarkers from patients exhaled breath before and after surgical resection of their tumors. The silicon microchip is consisting of silicon substrate with

2-(amino-oxy)- N, N, N trimethylethanammounium (ATM) iodide coat to trap carbonyl compounds in exhaled breath by means of oximation reactions. The sensitivity of this method is in the order of nmol/l, but it is not selective. E-nose techniques have been also applied to detect lung cancer from the exhaled breath with the sensitivity ranging from 71 to 96% and specificity from 33 to 100%. However, in most cases the results were not validated (Zieba et al. 2001). In one study, Bajtarevic et al. (2009) combined the exhaled breath data with clinical parameters and to improve the accuracy but not valiated. The method we proposed here is also in e-nose category. More than 1000 trace VOCs have been found in human breath (Hanna et al. 2019). It was found that tumorous and nontumorous cells, immune cells, and infectious agents may all contribute to the production of VOCs. In spite of the promising advances, the lack of normalization and standardization has led to significant variations in the VOC profiles and/or concentrations between the different studies reported. In an extensive study, Hakim et al. (2012) compared LC VOCs from 5 sources: cancer cells, blood samples, breath samples, urine, and saliva samples. They narrowed down the VOCs to find final 36 VOCs which were divided into seven compound families: hydrocarbons: alkanes, branched-chain alkanes, and branched-chain alkenes, primary and secondary alcohols, aldehydes and branched aldehydes, ketones, esters, nitriles, and aromatic compounds. Total of nine biomarkers were selected from different categories (Table 1). Pentane and heptane, from hydrocarbon category, were found in the breath of LC patients and/or in the headspace of LC cells.

Table 1 List of VOC biomarkers and their relevance to LC

Name	Compound family	Vapor pressure at RT	Ref
Butyraldehyde	Aldehyde	Found in tobacco smoke	Viale (2020)
Tetrahydrofuran	heterocyclic compound	Causes LC	Kinsinger et al. (2017)
Acetonitrile	Nitrile	SM > non-SM SM > LC Decrease in LC cells Cancer smoker and cancer ex-smoker	Lokhandwala et al. (2017), Hakim et al. (2012)
Heptane	Hydrocarbon	COPD > HC, SM > NSCLC	Nakhleh et al. (2017)
Hexanal	Aldehyde	LC > SM NSCLC > HC LC > HC SCLC > NSCLC Decreased in LC cells	
Benzene	Aromatic	Decreased SM > Non-SM SM > LC SM > NSCLC > HC	
Pentane	Hydrocarbon	Cancer cells	
2-Butanone	Ketone	LC > SM	Nakhleh et al. (2017)
Furan	Aromatic	Cancer smokers only	Hakim et al. (2012), Nakhleh et al. (2017)

LC lung cancer, SM smokers, Non-SM Non-smokers, NSCLC Non-small cell lung cancer, SCLC Small cell lung cancer, HC healthy control, COPD chronic obstructive pulmonary disease

Pentane was found in cancer cells while heptane found in the breath of LC patients. The concentration of 2-Butanone in exhaled breath of LC patients stages II through IV was significantly higher than in exhaled breath of patients with stage I (Fu et al. 2014). Aldehydes in tobacco smoke are saturated compounds, such as formaldehyde, acetaldehyde, propionaldehyde, and butyraldehyde, and unsaturated compounds, such as acrolein and crotonaldehyde (Filipiak et al. 2008). Benzene and furan were not found in the smoker cancer patients and mainly were found in the breath of non-smoker cancer patients. 2-Butanone was found elevated in the breath print of non-smoker cancer patients (Buszewski et al. 2007, 2012). Another study showed that lung cancer is associated with elevated level of exhaled pentane, which most probably attributed to an excess lipid peroxidation burden caused by cancer (Crohns et al. 2009).

2 Materials and methods

We previously reported an electrochemical gas sensor with glassy carbon electrode as the substrate (Emam et al. 2018). Later, silicon was substituted by the glassy carbon because in order to connect the sensor to other electronic components. A thin layer of chromium was sputtered on the silicon substrate to enhance the conductivity of silicon. Reduced graphene oxide was achieved by chemical reducing agent that was explained step by step (Emam et al. 2019a) and doped with Prussian blue to boost the sensitivity.

Then, a thin layer of molecularly imprinted polymer (MIP) is formed through the cyclic voltammetry through a 3-electrode system: working electrode, counter electrode, and a reference electrode. Platinum mesh was used as the counter electrode. Ag/AgCl reference electrode were purchased from Alfa Aesar and the sensor surface was the working electrode. Butyraldehyde, Tetrahydrofuran, Acetonitrile, heptane, hexanal, benzene, pentane, 2-butanone, and furan were purchased from Sigma Aldrich.

The electrochemical polymerization procedure starts with mixing pyrrole with the 0.5 mM of template molecule (a VOC biomarker) in aqueous solution of phosphate buffer solution (PBS). The cyclic voltammetry to deposit the polymer was carried out for 20 cycles with a scan rate of 50 mV/s, from -0.35 to +0.85 V vs. Ag/AgCl reference electrode. The MIP layer was achieved by washing by ethanol and removing the template molecule that led to cavities with the exact size and shape of the template molecule. Previously, this sensor that inherits the sensitivity of graphene and selectivity of molecular imprinting polymer was applied toward sensing biomarkers of Alzheimer's disease in the exhaled breath (Emam et al. 2018, 2019a, 2020) and detect explosives at the part per million level (Emam et al. 2019b).

Resistance changes of the sensors are so small; therefore, a precise resistance measurement technique is required. Measuring the resistance through ac signal amplification and conversion to the constant dc voltage at the output is utilized instead of conventional dc resistance measurement to improve the accuracy of the system to 10 milliohm resistance changes. The measured resistance is converted to digital domain and transmitted to the phone application for the post-processing and conversion to the exhaled breath concentration.

The as-designed on-board lock-in amplifier consists of a pre-amplifier, an AD630 based signal mixing block and an OP27 based low-pass filter. After the pre-amplification, the signal picked up at the output of the resistor ladder was mixed with the generated reference signal with same frequency, inducing a doubled frequency component and a DC component, the amplitude of each frequency component is determined by that of the picked-up signal of resistor ladder and hence reflect the tiny resistance change. The mixed signal is then fed into a low-pass filter block to extract the DC indicator signal, the time constant of this block is carefully chosen to optimize the frequency selectivity and the responding speed. The preliminary test data showed that by inserting a 1 KOhm pull-up resistor series connected with

Fig. 1 **a** the 4-layer structure of the gas sensor with electrical contacts to be connected to the PCB. **b** sensing mechanism of the sensor: as a template molecule traps inside the MIP layer, the extra electron is transferred to the graphene layer and causes the resistance change of graphene layer, which will cause the antenna's impedance to change

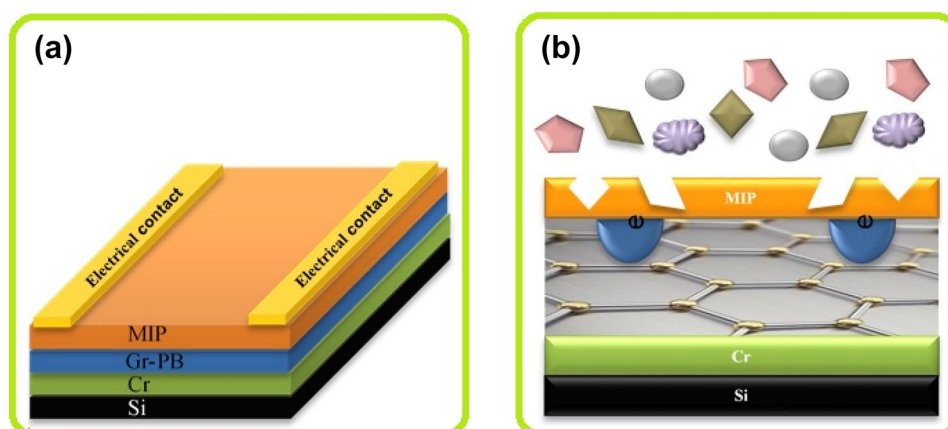
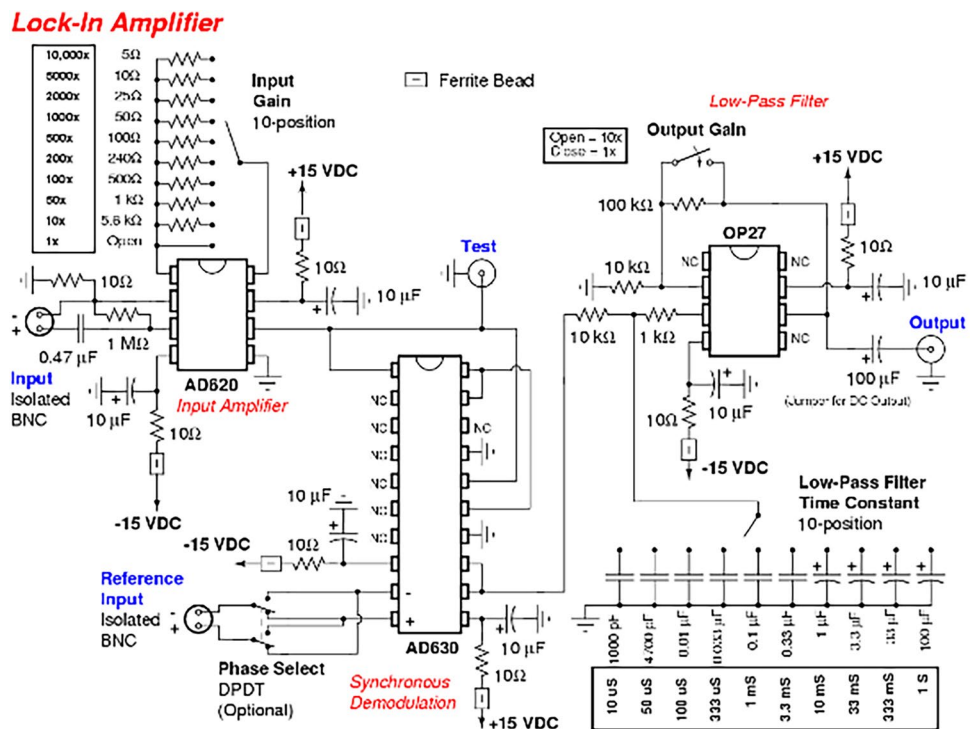


Fig. 2 Electronic components. The lock-in amplifier measures the resistance of the sensors & send the data through Bluetooth module in ESP32 microcontroller unit. The detail circuit of the lock-in amplifier is shown



the sensor, the lock-in amplifier can identify a 0.1Ω change in the sensor part when biased at 600 mV_{pp}, 23 kHz.

3 Results and discussions

Figure 1 shows the sensor layers and the sensing mechanism of the sensor. The gas-sensing mechanism is generally described as electron donors or acceptors. When a template molecule is trapped in the MIP layer, an extra electron transfers to the graphene layer and changes the resistance of the graphene.

The electronic system consists of three main blocks: ESP32 as a microprocessor, lock-in amplifier and the switching circuit. Bluetooth low energy is one of the best options that applies to the healthcare applications due to interoperability, low power operation, communication with smartphone, compatibility, etc. The utilized Bluetooth controller is able to provide 10 dBm output power which is suitable for the communication in the range of 10 m. ESP32 is the microprocessor chosen here due to a couple of features such as Bluetooth module, low power consumption and built-in analog to digital converter with 10 bits resolution. The ESP32 works as the heart of the whole system by providing

Fig. 3 The proposed lung cancer diagnosis device. **a** a patient blow into the replaceable mouthpiece and the results will be shown on his/her smartphone instantly, **b** the mobile application that graph the data during the test, and **c** the exploded view of the proposed lung cancer diagnosis handheld device

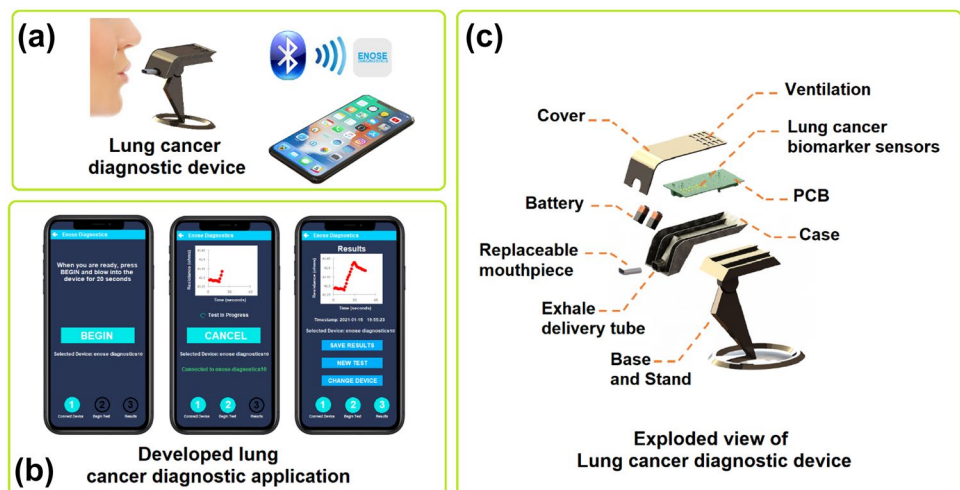
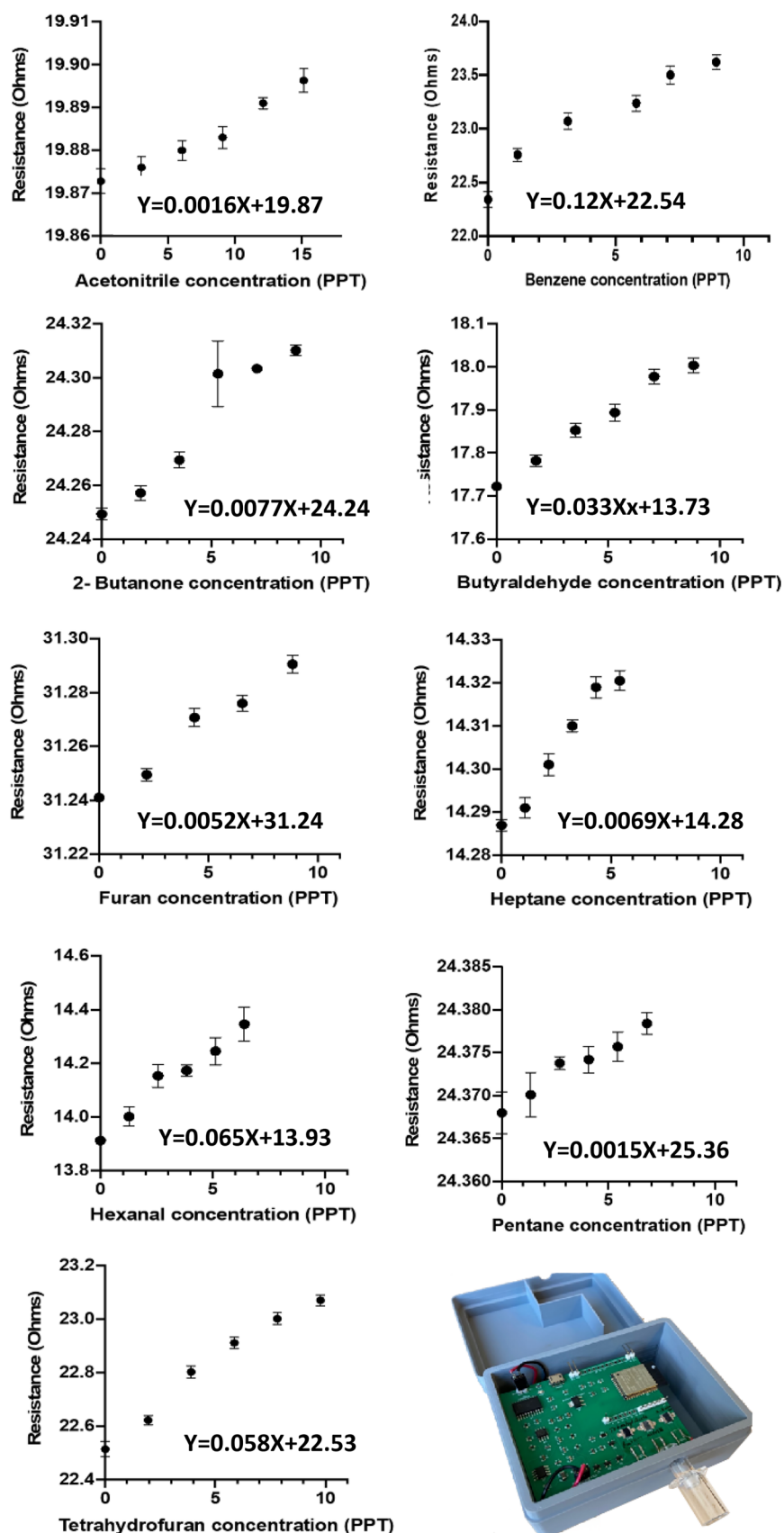


Fig. 4 Testing results of the sensors. Nine sensors were fabricated for nine biomarkers of lung cancer in the exhaled breath. These sensors were tested against their respective chemical in the range of 1–20 part per trillion (ppt) of the concentration. The sensors showed linear behavior toward sensing their respective template molecule



five main functionalities. It provides the pulse and reference signals required for the lock-in amplifier. Secondly, the built-in analog-to-digital (ADC) converts the analog signal from the lock-in output to the digital signal to be prepared for the transmission. Thirdly, four different signals are generated to control four complementary metal-oxide semiconductor (CMOS) transistors switches. The last one is Bluetooth module for transmitting the data to the phone application. The whole system schematic is shown in Fig. 2. ESP32 is programmed to generate six signals, two for the lock-in amplifier performance and four timing signals for the transistors switching operation. The input of lock-in amplifier is high when the all switches are on, therefore the first transistor (Vctrl1) is utilized for the circuit protection to reduce the output voltage of the lock-in amplifier.

The first switch is always on unless otherwise the whole system is turned on through the application, then the other switches are turning on every ten seconds. Lock-in amplifier is designed based on the low noise high precision amplifier that provides the high accuracy of the resistance measurement by comparing two input pulses and the output dc voltage is proportional to the input amplitude coming from the output of the switching blocks. Therefore, any amplitude changes in the resistor ladder will be interpreted to the output dc voltage changes. The lock-in frequency of operation is 23 kHz. The whole system is fabricated on the printed circuit boards (PCB) including the lock-in, ESP32 and the switching circuit integrated to each other. The whole PCB area is 65 mm × 70 mm and the thickness of 1.6 mm on FR4 substrate.

Nine sensors were fabricated and tested for the nine biomarkers selected. Three devices were fabricated and tested. Each device has 3 spots for the sensors and is able to measure the resistance of each sensor at the time and send the data to a smartphone. Each sensor was tested with its template molecule at a very low concentration (part per trillion). The vapor pressure of the selected VOC biomarkers is very high at room temperature, about 1 atm. So, they had to be diluted 10^{-12} times to reach ppt level. Such a sensitive sensor is reported for the first time. The state-of-the-art gas sensors reported so far are sensitive in the order of part per billion. The testing was performed in a sealed chamber under the hood. The results are graphed in Fig. 3. The resistance change showed linear behavior with the concentration change. So, it can be easily predicting the resistance change for higher/lower concentrations. The nine novel biomarkers along with microcontroller and the lock-in amplifier were placed in a box which was fabricated with 3D printing. The sensors were placed directly on the way of the VOCs and a ventilation was utilized for the case so the exhaled breath can find a way out after being sensed by the sensors. A disposable blow tube was also designed and fabricated for each test. At the start of the sensing operation, we send the

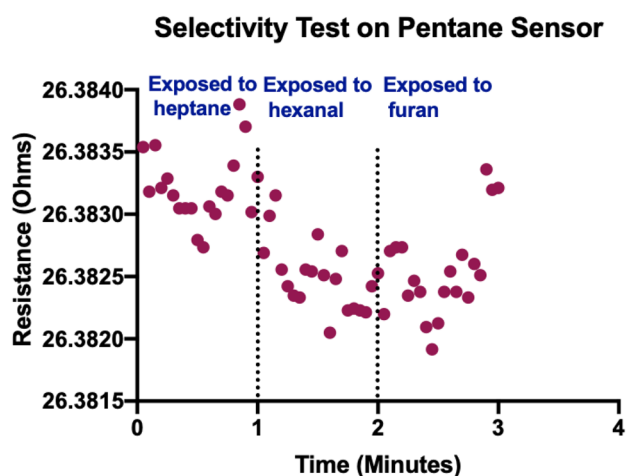


Fig. 5 Selectivity test: the pentane (C_5H_{12}) sensor was tested against heptane C_7H_{16} , hexanal $C_6H_{12}O$ and, furan C_4H_4O for 1 min each. So, the pentane sensor was exposed to each of other VOCs for 1 min while the resistance of the pentane sensor was being monitored. As shown in the graph, the resistance change is negligible

sensing command through the mobile application so that the ESP32 can start collecting the readings of the sensors. After the reading process is completed, the ESP32 sends back the readings of the sensors to the mobile application. The readings are basically the voltages across the sensors which are further processed in the mobile application to extract their corresponding resistance and hence extract the corresponding concentration. As evident from Fig. 4, a good linearity is achieved between the resistance variation vs. the concentration.

For the selectivity test, the pentane sensor was selected. The structure of pentane is C_5H_{12} . Heptane C_7H_{16} , hexanal $C_6H_{12}O$ and, furan C_4H_4O were chosen for the selectivity test because they have similar structure as pentane. Each of heptane, hexanal, and furan were exposed to the pentane sensor for 1 min while the resistance was being monitored. As shown in Fig. 5, the resistance change was negligible during the 3 min measurement, meaning that the pentane sensor did not pick heptane, hexanal, or furan.

4 Conclusion

A novel device was designed and fabricated to detect VOC biomarkers of lung cancers. This device is hand-held and is capable of communicating with a smartphone through Bluetooth. The device has 3 spots for 3 sensors and can measure the resistance of each sensor at the time. The circuit has been fabricated and tested with the sensors and a case was manufactured with 3D printing. Nine sensors for nine biomarkers of the lung cancer in the exhaled breath were fabricated and tested. The sensors were tested against the

pure reagent over the ppt concentration which is 3 orders of magnitude better than state-of-the-art sensors reported. All the VOCs have high vapor pressure at room temperature, so they were diluted a few times and injected into a sealed chamber with the size of 0.031 m³. The dilution process involved injecting 1 µl of the chemical into a 1L container and then taking 1 µl of the gas inside the container and inject into the sealed chamber. Since the dilution process was not performed with proper equipment, there is a high possibility of the chemicals being absorbed by the container or evaporated from the unvacuumed container. The results show a highly linear relationship between the resistance and the concentration. However, the resistance change for all the sensors are not consistent which is an evidence that the dilution process was not performed perfectly. The pentane sensor was also tested against structurally similar VOCs: heptane, hexanal, and furan. The selectivity test showed that the pentane sensor does not randomly react, it only reacts to one specific molecule which is pentane. This result can be generalized for other sensors as well. This device will be tested toward the exhaled breath of healthy control and lung cancer patients at Dana Farber Cancer Institute in Boston MA. If successful, it can be a game-changing to diagnose lung cancer instantly, cost-effectively, non-invasively with relatively high accuracy.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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