Lung-Cancer-Related Exhaled Breath Biomarkers

Subjects: Biochemical Research Methods

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Lung cancer has been studied for decades because of its high morbidity and high mortality. Traditional methods involving bronchoscopy and needle biopsy are invasive and expensive, which makes patients suffer more risks and costs. Various noninvasive lung cancer markers, such as medical imaging indices, volatile organic compounds (VOCs), and exhaled breath condensates (EBCs), have been discovered for application in screening, diagnosis, and prognosis. However, the detection of markers still relies on bulky and professional instruments, which are limited to training personnel or laboratories. This seriously hinders population screening for early diagnosis of lung cancer. Advanced smartphones integrated with powerful applications can provide easy operation and real-time monitoring for healthcare, which demonstrates tremendous application scenarios in the biomedical analysis region from medical institutions or laboratories to personalized medicine.

smartphone volatile organic compounds

1. Introduction

Lung cancer (LC) is the most commonly diagnosed cancer, as well as the leading cause of cancer death in both sexes around the world [1]. Unfortunately, more than half of lung cancer patients are diagnosed at an advanced stage rather than being controlled at a more treatable status, due to the physiological symptoms of lung cancer being difficult to find in the early stages. The 5-year survival rate can be improved from 20% to 70% if lung cancer is discovered at stage I [2][3]. Effective methods for early and accurate diagnosis of lung cancer are urgently needed.

Lung cancer, as a chronic disease, develops over a long period of time. The diversity and complexity of tumor biological behavior lead to gradual changes in metabolism and genome to a certain extent during carcinogenesis. To date, thousands of markers related to lung cancer have been studied, identified, and applied to determine stages of cancer progression and track the carcinogenesis process of lung cancer. Biomarkers of metabolism involving VOCs, proteins, and genes can reflect cellular, biochemical, and molecular (proteomic, genetic, and epigenetic) alterations and help recognize and monitor normal or abnormal biological processes, which can be applied in the screening, diagnosis, evaluation of treatment, and monitoring of recurrence of lung cancer. Thus, metabolic and genetic biomarkers may offer a way to track, monitor, and evaluate the carcinogenesis process in a more precise way [4][5][6]. Physical characteristics such as shape, density, and position of nodules in lung tissue imaged by chest X-ray, computer tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) have been regarded as effective means of cancer in diagnosis [7]. However, these conventional

instruments are relatively expensive, bulky, and limited to trained personnel, which greatly constrains their application in the field of population screening for early diagnosis. The development of novel technologies such as breath biopsy will allow researchers to detect cancer in the early stages and better identify the process of tumor cell growth and cellular metabolism. Detection of biomarkers in exhaled breath is one of the most promising methods due to its high-throughput detection, low cost, and noninvasiveness. According to different collection procedures, exhaled breath can be divided into a gas phase that is made of VOCs and a liquid phase, which is the condensate part of the breath containing both volatile and nonvolatile compounds [3][8][9]. Some techniques, such as gas/liquid chromatography (GC/LC) and mass spectrometry (MS), have been shown as a gold standard [10] in detecting VOCs and EBCs carried on human breath. However, despite significant improvements in sensitivity and accuracy, chromatography and spectrometry still have the aforementioned demerits, which hinders the wide range of application.

Recently, novel technologies based on smartphones have been developed to satisfy the demand for low cost, miniaturization, and easy operation. The smartphone is a highly integrated intelligent device that plays an irreplaceable role in the daily life due to its robust function, including a high-resolution camera, complementary metal-oxide semiconductor (CMOS) sensors, Bluetooth, near-field communication (NFC), universal serial bus (USB), and so on. Thus, as an ideal handheld device, a smartphone can provide a more convenient and fast response for healthcare detection. For example, smartphone-based electronic nose sensors, including quartz microbalances, surface acoustic wave sensors, and gold nanoparticles, have displayed great improvement in the analysis procedures for early diagnosis of diseases [11]. In addition, combined with smartphones, immunoassay analysis for the detection of genes and epigenetic markers such as DNA methylation and microRNAs in EBCs is also a promising method for lung cancer diagnosis [12]. These diagnostic procedures show high, broad relevance. This entry discusses lung-cancer-related biomarkers in exhaled breath and smartphone-based methods of detection, providing an overview of the recently developed technology for breath biopsy that can likely be utilized in future clinical practice. **Figure 1** summarizes the common collection methods for VOCs and EBCs, both in the laboratory and commercial platforms, focusing on smartphone-based detection technologies and the sophisticated function of smartphones for use in detection procedures.

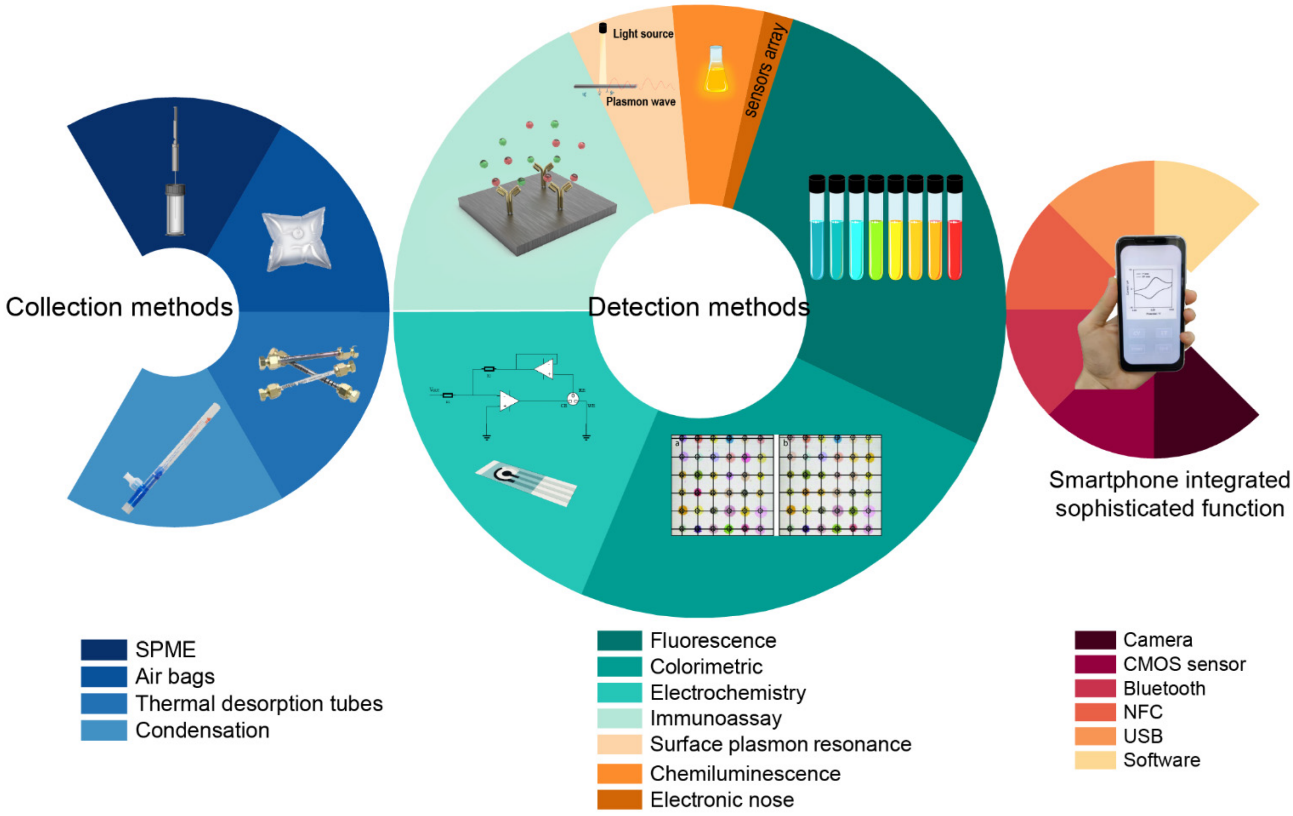

Figure 1. Schematic representation of smartphone-based biomarker detection platforms from sample collection to detection. Pie charts representing proportions of smartphone-based detection methods with different technologies.

2. Biomarkers in VOCs and EBCs

2.1. Breath Sampling and Preconcentration

Exhaled breath is composed of air from the mouth, nasal cavity, alveoli, and part dead space. Biomarkers associated with different diseases are produced in different locations. Exhaled breath biomarkers that have been fully exchanged in the lung–blood circulatory system will be comparatively accurate in their reflection of lung diseases and metabolic diseases. Although there are high-sensitivity gas sensors [13] that have achieved rapid online detection of components in exhaled breath, the most accurate detection of exhaled breath components still requires enrichment before detection and analysis. Therefore, the collection and storage of respiratory samples have an impact on subsequent procedures. However, there is still no standardized sampling protocol for collecting patient exhalation. In addition, the concentration of exhaled breath markers is between the volume fraction ppb and ppt. This concentration level is commonly lower than the limit of detection of equipment during direct detection of exhaled breath. Furthermore, the complete elimination of gas from unexchanged dead space and the oral and nasal cavities is still a challenge.

Thus, researchers have mostly chosen to collect late expiratory samples to eliminate dead space and have used polymer bags as collecting sample containers [14] due to the advantages of low cost and easy operation. Normally, subjects are asked to take a deep breath, and the first collected breath is discarded. Additionally, some researchers

stipulated strict sampling procedures. Chang et al. [15] asked subjects not to eat, smoke, brush their teeth, or gargle for a minimum of 2 h before sampling. Ambient air is another interference that can greatly alter analysis results. Di Gilio et al. [16] required that volunteers waited in the room for at least 10 min before breath collection so that equilibrium could be created between the lungs and ambient air.

Owing to the low concentration of VOCs in exhaled breath [17], preconcentration is commonly used to improve the accuracy and sensitivity of detection. Preconcentration methods include solid-phase microextraction (SPME), thermal desorption enrichment technology, needle trap microextraction (NTME), and so on. In essence, most methods use the force (such as van der Waals forces) between adsorption materials and gas molecules at the microscopic scale. The flexible use of these enrichment methods can not only eliminate more than 90% of the water vapor in exhaled breath but also greatly enrich the target markers by 10- to 100-fold. SPME is easy to use and widely used in many environmental, clinical, and biological analyses. Normally, SPME fibers are coated with various polymers such as polydimethylsiloxane, divinylbenzene, polyacrylate, and polyethylene glycol [18] to absorb analytes within the ppb range. Thermal desorption enrichment technologies mainly rely on adsorption materials. The adsorbents used in breath testing include carbon nanotubes, Tenax® TA, carbon black, and graphene. Due to its low breakthrough volume for water and high efficiency in the adsorption of volatile gas molecules, Tenax® TA is widely used in expiratory enrichment equipment. Tenax® TA is a 2,6-diphenyl furan resin polymer with a structure of porous microspheres that have a large specific surface area to adsorb target substances effectively. In addition, the filling of layered adsorbents can simultaneously concentrate multiple types of gas markers. The filled adsorbent tube needs to be heated at high temperatures in a flow of inert gas before use. It is worth noting that different materials have different desorption temperatures, and specific chemicals can be preserved by this technique, while others cannot [19].

EBC collection realized by condensing exhaled breath in a container in a low-temperature environment is easier than VOC sampling. Normal exhalation is saturated with water vapor at a temperature of 37 °C. When the external temperature is 0 °C and −10 °C, 89.1% and 93.7% of water vapor are condensed, respectively. Airway lining fluid, proteins of small relative molecular mass, and volatile compounds are exhaled together in quiet breathing. When the temperature is reduced, nonvolatile substances can condense into EBCs.

2.2. Biomarkers in VOCs

More than 1000 volatile organic compounds (VOCs) have been identified in human breath [20]. Lung-cancer-correlated VOCs have been sought in case–control studies and verified by cohort studies. To date, seven categories of VOCs have been found to serve as biomarkers of lung cancer in exhaled breath. The categories are alkanes, alkanols, aldehydes, ketones, lipids, nitriles, and aromatics. Researchers thought that alkanes such as ethane and pentane were generated by the lipid peroxidation of polyunsaturated fatty acids in cell membranes [21][22][23]. Breath methyl alkanes may also be products of the same process [24]. The first report on exhaled VOCs in lung cancer dates back to 1985, when Gordon et al. identified that several VOCs in exhaled breath were associated with lung cancer [25]. In 1999, Phillips et al. used a combination of 22 VOCs in breath samples to distinguish between patients with and without lung cancer [26]. Poli et al. demonstrated that a combination of 13

VOCs could be used to correctly classify lung cancer patients, chronic obstructive pulmonary disease (COPD) patients, asymptomatic smokers, and healthy subjects into defined groups [27]. Additionally, it is worth noting that some pulmonary diseases, especially COPD or emphysema, could be a major risk factor for lung cancer. This is because COPD and lung cancer share common risk factors such as smoking [28]. Wehinger et al. reported that the concentration of mass-to-charge ratios 31 and 43 were increased in lung cancer patients in a study using proton-transfer-reaction mass spectrometry (PTR-MS) [29]. **Table 1** summarizes the discovery of VOC markers related to lung cancer.

Table 1. Reported exhaled VOC markers for the early screening of lung cancer.

Years	Author	Collection Method	Sample	VOCs
1985	Gordon [25]	Tenax GC sorbent cartridges	Expired breath	Acetone, 2-butanone, n-propanol
1999	Phillips [26]	Sorbent trap	Alveolar breath	Styrene, 2,2,4,6,6-pentamethylheptane, 2-methylheptane, decane, n-propylbenzene undecane, methyl cyclopentane, 1-methyl-2-pentylcyclopropane, trichlorofluoromethane, benzene, 1,2,4-trimethylbenzene, isoprene, 3-methyloctane, 1-hexene, 3-methylnonane, 1-heptene, 1,4-dimethylbenzene, 2,4-dimethylheptane, hexanal, cyclohexane, 1-methylethenylbenzene, heptanal
2005	Poli [27]	Teflon® bulb; SPME	Mixed expiratory samples	Isoprene; methylpentane; pentane; ethylbenzene; xylenes; trimethylbenzene; toluene; benzene; heptane; decane, styrene; octane; pentamethylheptane
2007	Wehinger [29]	Tedlar® bags	Alveolar breath	Formaldehyde, isopropanol
2009	Bajtarevic [30]	Tedlar® bags	Mixed expiratory and indoor air	Isoprene, acetone, methanol; 2-butanone, benzaldehyde, 2,3-butanedione, 1-propanol, 2-butanone, 3-hydroxy-, 3-butyn-2-ol, butane, 2-methyl-, 2-butene, 2-methyl-, acetophenone, 1-cyclopentene, methyl propyl sulfide, urea, tetramethyl-, n-pentanal, 1,3-cyclopentadiene, 1-methyl-, 2-butanol, 2,3-dimethyl-, isoquinoline, 1,2,3,4-tetrahydro-, undecane, 3,7-dimethyl-, benzene, cyclobutyl-, butyl acetate, ethylenimine, n-undecane,
2010	Fuchs [31]	Mylar sampling bag	Alveolar breath	p-Cymene, toluene, dodecane, 3,3-dimethylpentane, 2,3,4-trimethylhexane, (1-phenyl-1-butenyl)benzene 1,3-dimethylbenzene, 1-iodononane, [(1,1-dimethylethyl) thiol]acetic acid, 4-(4-propylcyclohexyl)-4′-cyano [1,1′-biphenyl]4-yl ester benzoic acid, 2-amino-5-isopropyl-8-methyl-1-azulenecarbonitrile, 5-(2-methylpropyl)nonane, 2,3,4-

Years	Author	Collection Method	Sample	VOCs
				trimethyldecane, 6-ethyl-3-octanyl 2-(trifluoromethyl)benzoate, p-xylene, and 2,2-dimethyldecane
2010	Song [32]	Tedlar® gas bags; SPME	Mixed expiratory samples	1-Butanol and 3-hydroxy-2-butanone
2011	Ulanowska [33]	Tedlar® bags; SPME	Alveolar breath	Ethanol, acetone, butane, dimethyl sulfide, isoprene, propanal, 1-propanol, 2-pentanone, furan, o-xylene, ethylbenzene, pentanal, hexanal, nonane
2012	Buszewski [34]	Tedlar® bags; SPME	Alveolar breath	Butanal, ethyl acetate, 2-pentanone, ethylbenzene, 1-propanol, 2-propanol
2015	Kumar [35]	Nalophan bag	Mixed alveolar breath	Pentanoic acid; hexanoic acid; phenol; methyl phenol; ethyl phenol; butanal; pentanal; hexanal; heptanal; octanal; nonanal; decanal
2016	Schallschmidt [36]	Gas bulbs; SPME	Tidal breath	Propanal, butanal, decanal, butanal, 2-butanone, ethylbenzene
2017	Sakumura [37]	Analytic Barrier Bag	Alveolar breath	Hydrogen cyanide, methanol, acetonitrile, isoprene, 1-propanol
2019	Phillips [38]	Carbotrap C and Carbopack C	Alveolar breath	1,4-Butanediol, 2-pentanamine, 4-methyl-, 2-propanamine, 3-butenamide, 4-penten-2-ol, acetamide, 2-cyanoalanine, n-methylglycine, octodrine
2019	Li [39]	Tedlar® bags; SPME	End-tidal breath	Isopropanol, n-butanol, n-heptanol, n-hexanal, n-heptanal, n-decanal

Based on the aforementioned techniques, smartphones provide promising potential in lung-cancer-correlated VOCs such as alkanols and ketones. For instance, Salimi and colleagues [40] introduced a smartphone-based chemiresistive device using a zinc oxide nanosheet that could detect diethyl ketone (LOD = 0.9 ppb), acetone (LOD = 4 ppb), isopropanol (IPA, LOD = 11 ppb), and other alcohols that are related to lung cancer. Most recently, a fluorescence-based smartphone platform was reported for real-time/on-site, sensitive, and quantitative visual detection of IPA in exhaled breath [41]. In this approach, red carbon dots (RCDs) were modified by a coenzyme (nicotinamide adenine dinucleotide, NAD^+) for fluorescence, as shown in **Figure 2**A. This NAD-dependent enzymatic reaction provided the electron transfer from IPA to NAD^+ and displayed continuous color changes from red to light blue, which could be detected and captured by a smartphone. The detection limit and recovery rate of the fluorescence-based smartphone platform were 8.34 nM and 90.65–110.09% (RSD ≤ 4.83), respectively.

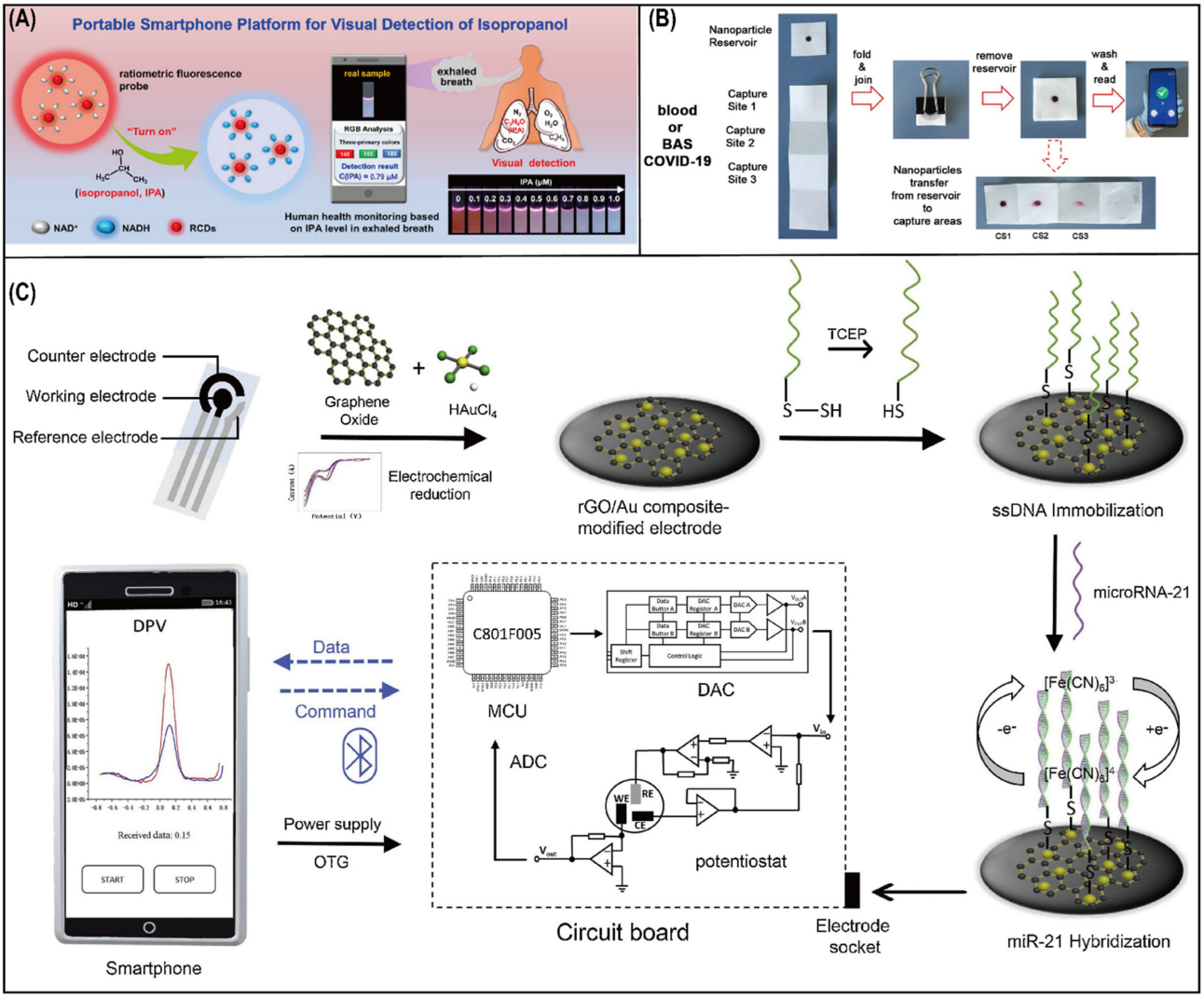

Figure 2. Schematic diagram for detection of IPA, IL-6, and microRNA using a smartphone. (**A**) Schematic diagram of the ratiometric fluorescence sensing system for IPA detection and RGB analysis of the fluorescent photo captured by the smartphone. (**B**) Diagrammatic representation of the paper immunosensor and analytical steps for detecting IL-6 in blood or respiratory samples (bronchial aspirate (BAS)) from COVID-19 patients. (**C**) Illustration of smartphone-based electrochemical biosensing system, including reduced graphene oxide/gold composite-modified electrode, circuit board, and smartphone with Android application.

2.3. Biomarkers in EBCs

EBCs also provide a convenient and noninvasive method for the diagnosis of lung cancer. EBCs, which are formed by cooling exhaled breath, contain low-volatility or nonvolatile markers that reflect the status of the airway lining fluid (ALF) environment [8]. Many biomarkers have been reported in EBCs. Carpagnano et al. indicated that the concentrations of inflammatory markers such as cytokines including interleukin-6 (IL-6) and vascular stimulating factor (endothelin-1) were significantly different in the EBCs of patients with lung cancer than controls, and the same difference between the various stages of NSCLC was also found. In addition to the cytokines mentioned

above, oxidative-stress- and lipid-peroxidation-related factors, including 8-isoprostane F2α, hydrogen peroxide (H2O2), etc., were also found in EBCs [42].

More interesting data have been acquired by researching genetic markers in EBCs. Microsatellite DNA alterations involving carcinogenesis and tumor progression are valuable as clonal markers for the detection of cancers. Carpagnano et al. demonstrated the possibility of studying microsatellite alterations (MAs) of 3p in the DNA of EBCs in patients with NSCLC. They concluded that MAs were significantly more frequent in NSCLC patients than in control subjects. In addition, epigenetics has become increasingly important in the early diagnosis and treatment of lung cancer. DNA methylation, histone modification, and long noncoding RNA (lncRNA) can all be used as biomarkers for lung cancer detection [43][44]. Han et al. showed that DNA methylation in EBCs is detectable, and that the DNA appears to be of lower airway or lung origin and has some association with lung cancer and smoking [45]. Epigenetic markers can be found in blood, serum, EBCs, phlegm, and alveolar analytes [46], reducing the invasiveness of sampling. Considering the integrality of this marker in the field of cancer studies, DNA analysis in EBCs seems to be a strong screening tool.

The detection of markers in EBCs is always performed using marked immunoassay technology for protein markers and polymerase chain reaction (PCR) for genetic markers. On the basis of a marked immunoassay, Zhang et al. developed a love-wave surface acoustic wave (LWSAW) immunosensor to measure proteins in EBCs. They reported a miniaturized platform that provides online detection of CEA in EBCs. The detection method adopted here was a sandwich immunoassay using AuNP–antibody conjugates with a gold-staining signal enhancement strategy. This platform displayed a good performance in the measurement of CEA concentrations, with a limit of detection of 1.25 ng/mL and a coefficient of determination (r^2) of 0.998 [47]. In addition, liquid chromatography–mass spectrography (LC–MS) is also very popular with many researchers [48][49]. However, some limitations impede the application of LC–MS in the fast analysis of exhaled breath. The complexity of operation and maintenance of LC–MS, especially in the preprocessing of samples, introduce a stringent requirement for operators. Additionally, the expensive cost and large size of this instrument might deter small institutions. Thus, smartphone-based LOC systems involving portability, low cost, easy operation, and relatively high sensitivity and specificity have attracted the attention of researchers.

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