Wearable breath analysis

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Biomarkers in breath can be related to certain diseases, which makes breath-based analysis a powerful diagnostic tool. Here we highlight milestones and remaining challenges for the broad clinical implementation of wearables for breath analysis.

Breath was first analysed by chromatography in the 1970s, demonstrating the presence of organic substances in exhaled breath. Since then, more than 3,000 volatile organic components (VOCs) have been identified in breath, which could be explored in medical applications[1,2]. However, except for breath analysers that measure alcohol levels, only few sensing devices have reached high technology readiness levels[2], and diagnostic application has been limited to chromatographic laboratory tests. Advances in material science, synthetic biology and engineering, in combination with an increase in interest in the diagnostics of infectious diseases from exhaled breath, have recently pushed the popularity of breath analysis.

Breath analysis as diagnostic tool

Breath analysis can be broadly interpreted as a two-step process: verifying the presence of diagnostic breath markers, and establishing a method of their detection. The identification of specific VOCs that are related to certain diseases or metabolic activities in so-called 'breath prints' has been established by clinical trials. Thus, breath analysis can be used as a non-invasive, qualitative diagnostic tool[2]. Although there is still no full consensus on how to interpret the measured biomarker levels to identify their association with diseases, inference of targeted concentrations in breath is not out of reach. Breath sampling offers a unique advantage compared to other non-invasive sampling methods, because the transportation of analytes from blood into the lungs bypasses complex transport mechanisms[2]. For example, the transportation of secreted molecules into the saliva and sweat depends on their dissociation constant, lipophilicity, pH, protein-binding affinity and ionizability, and thus can be much more complex than capillary diffusion through lung alveoli. Recent studies demonstrated a good correlation between antibiotic levels in exhaled breath condensates and blood, underlining this hypothesis[3].

Wearable breath sensors

The transition from laboratory-based measurement techniques to wearable biosensors is necessary to accelerate the translation of breath analysis. The former relies on the collection of a breath snapshot, typically in the form of an exhaled breath condensate, which is then analyzed by chromatographic and/or spectroscopic methods. However, these methods are limited by a lack of standardization in workflows, long turnaround times, high instrumentation costs and complex sample preparation. Alternatively, wearable sensors offer simultaneous sample collection and analysis. Wearables allow the collection of breath over a long period of time, providing real-time sampling and analysis, instead of a snapshot. In addition, biomarkers can be concentrated for the detection of small amounts, and differential physiological changes can be detected from an individual baseline.

Wearables in the form of face masks have been tested for the detection of hydrogen peroxide (a biomarker for several respiratory illnesses, such as asthma, chronic obstructive pulmonary disease and lung cancer) by electrochemical sensing[4], and for breath-condition monitoring (for example, respiration rate, cough and breath holding) by the integration of a self-powered pressure sensor[5]. The COVID-19 pandemic has further brought breath analysis to the front line as a method for detecting airborne-transmitted infectious diseases from virus-containing aerosols. For example, face masks with a lyophilized CRISPR sensor can detect nucleic acids of SARS-CoV-2 using conventional lateral flow assays[6], and an optical sniffer (colourimetric sensor array) can provide semi-quantitative analysis of COVID-19 severity (from very mild to severe) by using the relationship between colour patterns and the medical reports of patients, together with real-time polymerase chain reaction (RT-PCR) results[7].

Sensitivity

To realize the clinical potential of wearable breath analysis, several challenges need to be addressed. A variety of breath analytes (around 3,500) have been identified, including exogenous markers that are not physiologically produced; however, they typically occur at very low concentrations in breath (1,000–10,000 times lower than in blood)[2,8], depending on age, diet, smoking and medication, as well as the expiratory flow rate. Therefore, the sensitivity of wearable breath analysers has to be high to enable the detection of breath analytes. Concentrating target molecules is necessary, either on the wearable device or at the source (such as the lung and/or respiratory tract).

In wearable devices, breath can be segregated within a microfluidic system as a pre-concentration step (for example, μ-gas chromatography). In addition, the sensing element can be improved with synthetic biology-based bioassays (such as CRISPR–Cas-based systems, microbial enzymes and proteins) to augment sensitivity, or nanostructures can be implemented to increase the effective sensing area. Target molecules can also be concentrated at the source by stimulating metabolic activity; for example, volatile markers can be released in response to protease activity[9]. Alternatively, exogenous biomarkers can be expressed at the source through the exploitation of metabolic activities associated with a condition, such as lung cancer, or through the application of engineered living organisms[9]. For example, genetically engineered synthetic biomarkers can be designed that are specific for tumour-associated gene expression or dysregulated activities through the implementation of tumour-specific promoters that encode secretable reporters that are transcriptionally targeted to cancer cells[9]. Analyte reactivity can also be increased by chemical pre-treatment or through the formation of analyte–nanoparticle conjugates.

Selectivity

To measure specific analytes in a mixture of substances with similar chemical and/or physical properties, an antithetical design approach can be followed. The bottom-up approach aims for absolute selectivity
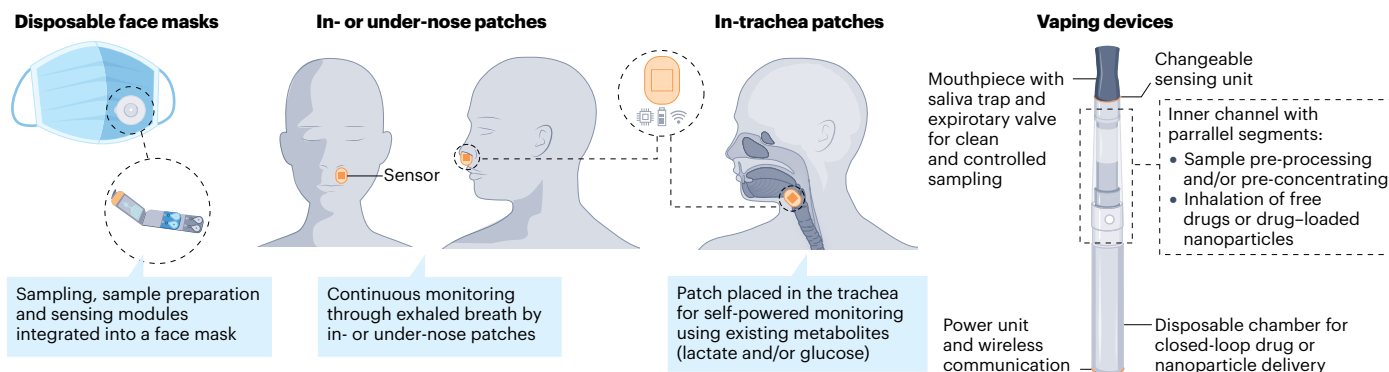
Fig. 1 | Wearables for breath sampling and analysis. Robust analysis can be achieved through the use of face masks integrated with sampling, sample preparation (if required) and sensing modules, in- or under-nose patches, in-trachea patches and vaping devices.

and starts with the selection of a well-defined target (bio)marker. The sensing technology is designed around that particular compound in an idealized lab environment. Measurement complexities owing to the analyte (the number of interfering substances with similar chemical properties) and environmental factors (for example, temperature and humidity effects can be subtracted by the addition of a blank as a reference system) are systematically increased to improve the generalizability of the measurement technique. The interaction strength between the target and receptor should be optimized to allow sensor replenishment for continuous measurement[8]. Synthetic breath biomarkers can help to improve the signal-to-noise ratio and enable optimization of analyte capture–release cycles for sensor regeneration. Similarly, synthetic biology-empowered bioreceptors (such as molecularly imprinted polymers or aptamers) enable controlled analyte capture and release (by changing surface charge, temperature or pH) and, thus, continuous monitoring[2].

In the top-down approach, an array of sensors is used to increase selectivity. Such sensing units need to be robust and apply a range of chemical interactions to capture different combinations of potentially informative compounds. The interpretation of results then relies on similarities or dissimilarities across measurements. Therefore, a large amount of data must be compiled and processed with the assistance of pattern-extraction models[1] to correlate the physiological status with the sensory fingerprint. However, the same subset of sensors can be stimulated by different analyte combinations, which makes it difficult to correlate patterns with certain conditions. Therefore, sensory patterns associated with certain health conditions could be observed even in the absence of physiologically relevant biomarkers. This dilemma can be overcome by equilibrating the complexity between the sensory problem, hardware and model architecture. Breath has a rich chemical composition of analytes, and a priori prediction of the relevant subset of analytes remains challenging. Therefore, multiarray sensing technology should be complex enough to respond to the variances in composition. The only known sensing unit that reflects such complexity are olfactory receptors, which could be incorporated into biohybrid sensors of wearable breath analysers[10]. Furthermore, the data must be processed with black-box models of similar complexity, such as deep-learning methods[1], which can be trained only with large volumes of data.

Sampling

The composition of breath is strongly influenced by the sample-collection method, which has not yet been standardized. The relative ratio of analytes changes with the selection of the breath portion (late or end-tidal breath), breathing patterns, sample contamination (for example, with saliva), mode of sample collection (on-line continuous or off-line discrete) and phase of the sample (vapour (gaseous) or condensate)[8].

Thus, end-to-end wearable design studies should select a sample-collection strategy that allows continuous access to the physiological state. For example, disposable face masks could integrate sampling, sample preparation (if required) and sensing modules. Alternatively, under or in-nose patches, or implants mounted to the respiratory tract (possibly self-powered using metabolites, such as lactate or glucose) could be applied. Sensor-integrated vaping devices could enable closed-loop drug delivery for theranostic applications (Fig. 1).

Ethical considerations

Ethical considerations related to wearable breath analysis include patient data collection, patient safety, liability, legal responsibility, user compliance, accessibility and equity, which need to be accounted for in the design and clinical application of wearable breath sensors[1,8]. Liability and patient safety are special concerns for wearables for theragnostic applications. Legal responsibility arises from the integration of patient data into data pipelines, and error or bias in the selection of model and training databases, which in turn determines the contribution of wearable output to the final diagnostic judgement. In addition, misuse of the device and lack of software updates, considering programming languages, libraries and medical cues of certain diseases, lead to liability concerns[1,8].

By addressing the remaining technological and ethical challenges, wearable breath analysis could become a complementary tool for distributed, preventive healthcare monitoring and transform our understanding of diagnostics.

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Competing interests
The authors declare no competing interests.