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Sensors for Breath Testing: From Nanomaterials to Comprehensive Disease Detection

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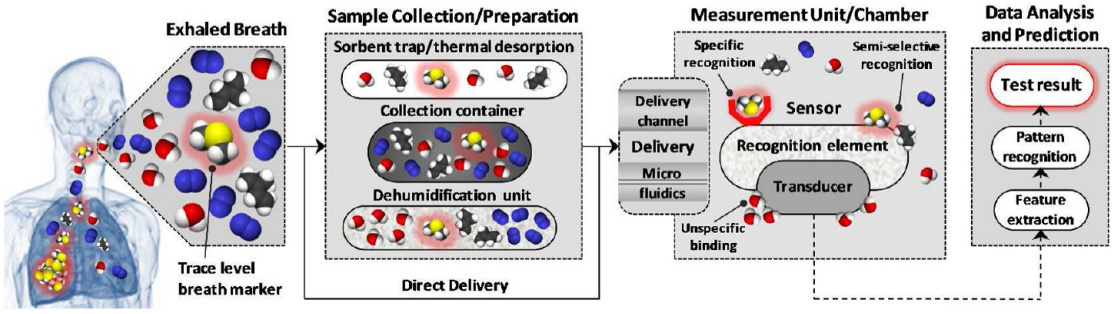

The analysis of volatile organic compounds in exhaled breath samples represents a new frontier in medical diagnostics because it is a noninvasive and potentially inexpensive way to detect illnesses. Clinical trials with spectrometry and spectroscopy techniques, the standard volatile-compound detection methods, have shown the potential for diagnosing illnesses including cancer, multiple sclerosis, Parkinson's disease, tuberculosis, diabetes, and more via breath tests. Unfortunately, this approach requires expensive equipment and high levels of expertise to operate the necessary instruments, and the tests must be done quickly and use preconcentration techniques, all of which impede its adoption.

Sensing matrices based on nanomaterials are likely to become a clinical and laboratory diagnostic tool because they are significantly smaller, easier-to-use, and less expensive than spectrometry or spectroscopy. An ideal nanomaterial-based sensor for breath testing should be sensitive at very low concentrations of volatile organic compounds, even in the presence of environmental or physiological confounding factors. It should also respond rapidly and proportionately to small changes in concentration and provide a consistent output that is specific to a given volatile organic compound. When not in contact with the volatile organic compounds, the sensor should quickly return to its baseline state or be simple and inexpensive enough to be disposable.

Several reviews have focused on the methodological, biochemical, and clinical aspects of breath analysis in attempts to bring breath testing closer to practice for comprehensive disease detection. This Account pays particular attention to the technological gaps and confounding factors that impede nanomaterial-sensor-based breath testing, in the hope of directing future research and development efforts towards the best possible approaches to overcome these obstacles. We discuss breath testing as a complex process involving numerous steps, each of which has several possible technological alternatives with advantages and drawbacks that might affect the performance of the nanomaterial-based sensors in a breath-testing system. With this in mind, we discuss how to choose nanomaterial-based sensors, considering the profile of the targeted breath markers and the possible limitations of the approach, and how to design the surrounding breath-testing setup. We also discuss how to tailor the dynamic range and selectivity of the applied sensors to detect the disease-related volatile organic compounds of interest. Finally, we describe approaches to overcome other obstacles by improving the sensing elements and the supporting techniques such as preconcentration and dehumidification.

Introduction

Detecting a disease through the smell print of a person's breath, via a signature of volatile organic compounds (VOCs), has been long recognized as having great potential as a rapid and noninvasive method for widespread screening and disease diagnosis.[1,2] Clinical trials have shown the possibility of using breath for detecting serious illnesses, such as different types of cancer, multiple sclerosis,

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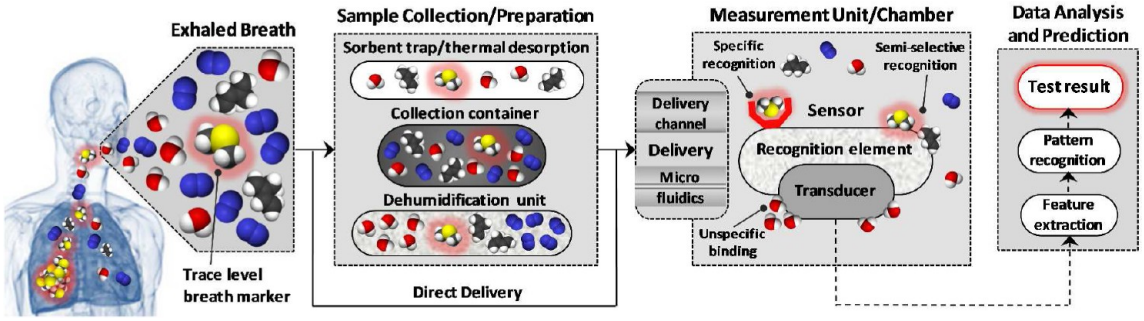

FIGURE 1. Overview of the processes involved in breath testing.

Parkinson's and Alzheimer's disease, tuberculosis, diabetes, and chronic kidney disease. Several reviews have covered important biochemical, clinical, and methodological aspects of breath testing,[1−4] while other reviews have highlighted considerable advances in gas and VOC sensing technologies.[4−6]

Important milestones have been reached in the field of breath analysis. Nevertheless, only few breath tests are currently practiced in the clinic.[1] This situation is primarily a consequence of the technological difficulties in the detection of trace amounts of disease-related VOCs within a complex exhaled breath sample. An additional reason for this situation is that the wide variety of implemented techniques causes a major standardization problem in the field. Part of these methods is still premature and, therefore, confined to research. The other part of the methods include established analytical approaches, such as mass spectrometry, but these tools suffer from high costs, complexity, and require trained personnel for their operation.[3,5]

In recent years, special attention has been given to methods incorporating nanomaterial based VOC/gas sensors (NMVSs) because they would enable the development of highly sensitive, rapidly responsive, and yet cheap detection systems.[6] These virtues could be attributed to the fact that nanoscale dimensions are associated with unique and controllable physical, chemical, and optical properties as well as with low cost fabrications. For instance, the dynamic range as well as the selectivity of the NMVSs can be tailored to accurately detect specific breath VOCs of a given disease.

Breath testing involves a complex multistep process in which each step has its own advantages and drawbacks with respect to the performance of the NMVSs (see Figure 1). With this in mind, we describe in this Account the benefits and implications of using NMVSs for breath testing by emphasizing the fundamental challenges, such as the need to detect trace amounts of VOCs while addressing unspecific interactions between confounding species and the nanomaterial sensing elements. We also suggest means to overcome these obstacles. By doing so, we wish to direct future research efforts in the field of nanotechnology, and NMVSs in particular, toward the development of comprehensive breath testing systems.

Nanomaterial-Based VOC and Gas Sensors

The implementation of nanotechnology in the field of chemosensors has increased in recent years, resulting in a growing number of related publications. Various nanomaterials have been utilized for VOC sensing elements, including nanoparticles and nanowires of different materials and carbon nanotubes. The nanoscale size of these building blocks provides them with several merits, such as large surface-to-volume ratio and unique chemical, optical, and electrical properties. The increased surface area of the nanomaterials provides highly active interfaces, thus increasing sensitivity and lowering the response and recovery times.[6] Additionally, the nanoscale size makes nanomaterials sensitive to localized entities of similar size, from small molecules to large macromolecules.

Nanomaterials are frequently used as highly sensitive transduction elements.[6] Common examples of transducers based on nanomaterials include field effect transistors (FETs) based on single-walled carbon nanotubes (CNTs)[7] (see Figure 2a) or nanowires of various materials (see Figure 2b),[8] nanoporous chemioptical materials,[9] chemiresistors based on films of monolayer capped metal nanoparticles (MCNPs),[10] random networks of single-walled CNTs,[11] and silicon nanowires.[12] Combining these nanomaterials with organic recognition elements result in transducers that can be utilized (i) as sensors with specific receptors (lock-and-key) that have high sensitivity (detection limits down to ppb_v and lower) and selectivity (see Figure 2a),[12]

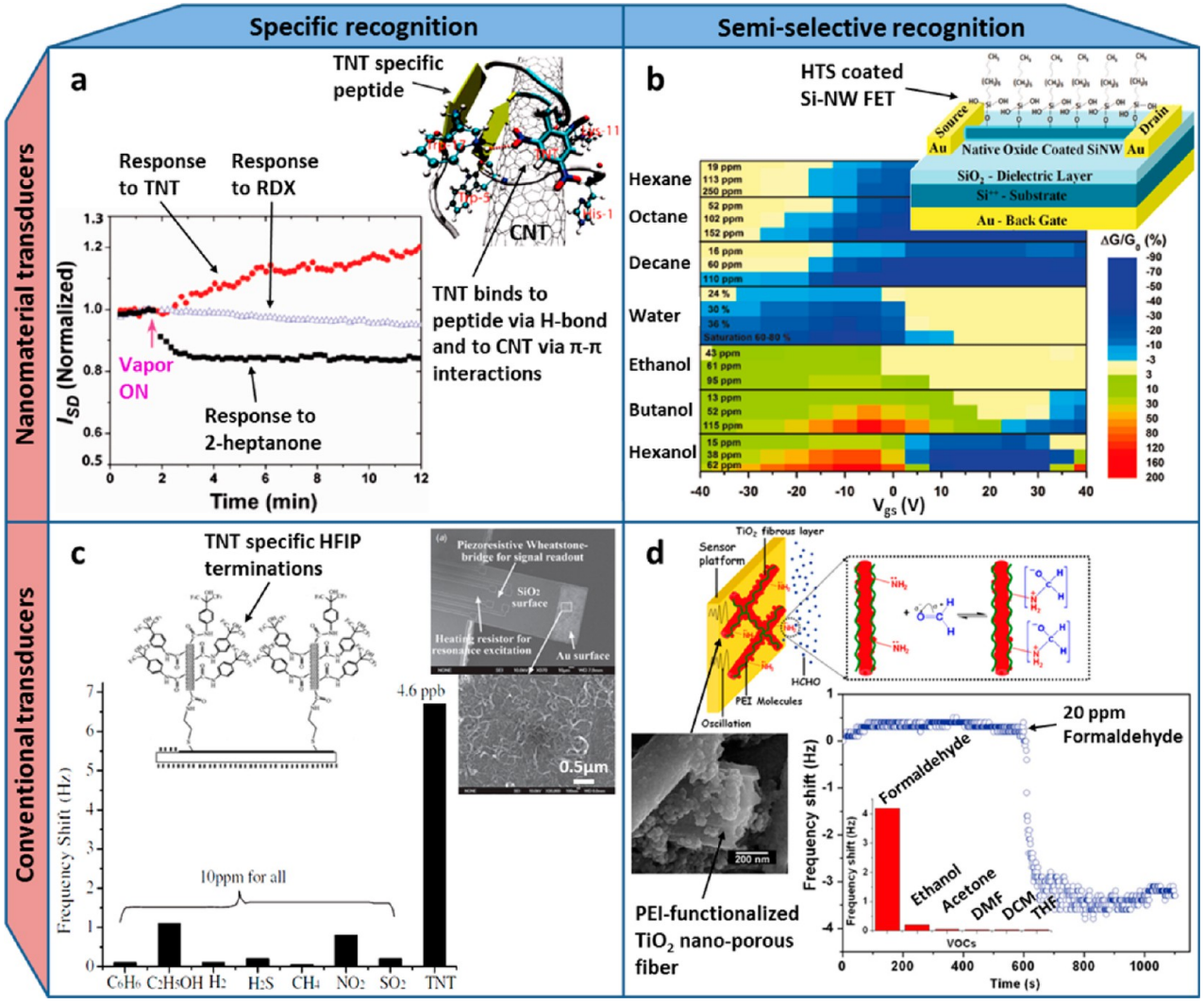

FIGURE 2. Examples for the four configurations of NMVSs: (a) Transducer based on a CNT FET coated with trinitrotoluene (TNT) specific binding peptides. Reprinted with permission from ref 7. Copyright 2010 American Chemical Society. (b) Transducer based on a Si-nanowire FET coated with an organic self-assembled monolayer of hexyltrichlorosilane (HTS) which interacts semiselectively with (nonpolar) alkanes. Reprinted with permission from ref 8. Copyright 2011 American Chemical Society. (c) Transducer based on a conventional microcantilever loaded with hexafluoroisopropanol (HFIP)-functionalized CNTs as a TNT-specific recognition layer. Reprinted from ref 15 © IOP Publishing. All rights reserved. (d) Transducer based on a conventional QCM oscillator coated with a sensing layer of polyethyleneimine (PEI)-functionalized TiO_2 nanoporous fibers with increased selectivity (semiselectivity) toward formaldehyde. Reprinted from ref 16, with permission from Elsevier.

or (ii) as semiselective sensors that are less sensitive (detection limits typically down to hundreds of ppb_v) and less selective but are more suitable for characterizing complex and unknown samples (see Figure 2b).[5,13,14] In order to achieve higher flexibility in the latter case, the combined responses of arrays of semiselective (that is, cross reactive) sensors are used to establish VOC-specific responses, by applying pattern recognition and classification algorithms. Such systems mimic the human olfaction system and therefore are often referred to as "electronic noses".[5,6]

Nanomaterials can also be used as highly sensitive recognition elements coupled with conventional well-established transducers, such as gravimetric transducers based on microcantilevers (see Figure 2c),[15] quartz crystal microbalance (QCM) (*see* Figure 2d),[16] surface acoustic wave (SAW),[17] or surface plasmon resonance (SPR) spectroscopy.[18] NMVSs based on conventional transducers can be divided into (i) sensors with specific receptor layers (see Figure 2c) and (ii) sensors with semiselective recognition layers (see Figure 2d). In the case of optical transduction a somewhat more direct measurement of the recognition element can be made. This case occurs when the nanomaterial recognition element is directly probed for the modulation of an optical property resulting from direct

interactions with the VOC.[5] In such cases, the sensors might be less affected by real world confounding factors, such as unspecific adsorption and condensation of water vapor.[19,20]

Some limitations exist alongside the many advantages of NMVSs. While the variety of available transducers is large and offers extremely high transducer sensitivities, only a handful of applicable molecular recognition approaches exist, especially for gas phase detection. This shortage mainly results from the complexity of immobilizing specific receptors on solid/gas interfaces without hampering their functionality. This gap must be minimized by directing research efforts toward the development of novel molecular recognitions that can mimic the bioreceptors and that can smoothly integrated with nanomaterials. Among the most prominent examples in this direction are the use of surface-immobilized macromolecular cavitands[21] and "cavity-like" nanoporous materials,[9] for which the recognition is based on "host−guest" interactions, as well as the use of nanomaterials decorated with biomaterials such as peptides[12] and single-stranded DNA oligomers[22] as VOC-specific receptors.

Nevertheless, high selectivity usually comes at the price of irreversibility in the interaction between the VOC and recognition element, which might impose lengthy recovery times and memory effects. This problem could be tackled by applying thermal cycles or UV radiation to the NMVS.[23] In case organic sensing materials are employed, special care should be taken to assure minimal degradation of the nanomaterial under high temperatures or UV radiation. An additional limitation resulting from the small surface area of nanoscale elements is the reduced probability of receptor/VOC interaction, which makes lengthy measurement times necessary.[24] However, this problem can be overcome either by incorporating sample preconcentration[5] or by enlarging the active surface area of the NMVS using 3D matrices of the recognition elements. Sensors based on random networks of carbon nanotubes (RN-CNTs) (see Figure 2a and c),[11] functionalized nanoporous TiO_2 fibers (see Figure 2d),[16] or MCNP films[13,14,25] present the use of such 3D matrices. Finally, the inherent nature of transducers to facilitate nonspecific interaction is problematic in the case of nanomaterial transducers because nanomaterials have exceptionally large surface-to-volume ratio. Consequently, the higher the sensitivity of the nanomaterial transducer, the more vital becomes the enhancement of its surface coverage using either the recognition layer or a protective passivation layer (or both).

Sensing Breath VOCs and Gases

The detection of a disease through the analysis of a breath sample requires the ability to sense disease-related abnormalities in the levels of breath VOCs (or gases) despite inherent variations in the levels of other unrelated (that is, confounding) VOCs. This strategy requires in-depth knowledge of the composition of breath and the different factors governing it. Generally, exhaled breath samples contain (in a decreasing order by volume) nitrogen, oxygen, carbon dioxide, water vapor, argon, and a variety of thousands of VOCs that appear mostly in parts per billion levels.[1,3] A major part of the VOC spectrum varies among different individuals while the rest of the VOCs could be found in all breath samples of a given population. Apart from rare cases, no specific VOC is uniquely found in the breath of diseased subjects. Rather, VOC(s) that can indicate a clinical state are the ones common for any breath sample but exhibit distinct levels with the disease. For example, a typical population of breath samples might contain around 3000 different VOCs in total.[1] However, the number of common VOCs found in the breath of all patients, which might be indicative of a given clinical state, ranges from only a few to tens of VOCs.[3] A common conception is that VOCs that do indicate the presence of a disease appear in breath through two main paths: (i) metabolic changes and oxidative stress associated with the disease induces VOC blood content changes, which are then expressed in breath following pulmonary material exchange in the lungs; (ii) specific VOCs generated by cells and tissues that are linked to the pathological state and are adjacent to epithelium tissues lining the respiratory system or the upper gastrointestinal (UGI) tract can be expressed in breath, in addition to the first path, through direct outgassing.[1,3] In both cases, the breath concentrations of these VOCs are low (mostly sub-ppm_v levels) compared to the total breath composition. Yet, according to numerous studies, the concentration range of such VOCs spans across several orders of magnitude, from ppt_v levels to ppm_v levels, with some clinical states reported to be associated with distinct breath odors detectable even by human olfaction.[1,3]

When aiming to recognize a disease-related breath print using NMVSs, the main considerations could be tailoring the sensor's dynamic range according to the breath concentrations of the target. An additional consideration could be tailoring the NMVS's specificity in order to lower the sensitivity to variations in the overall VOC background.[2] However, matching appropriate NMVSs to target VOCs of a given disease is not a simple task. Primarily, this task requires prior

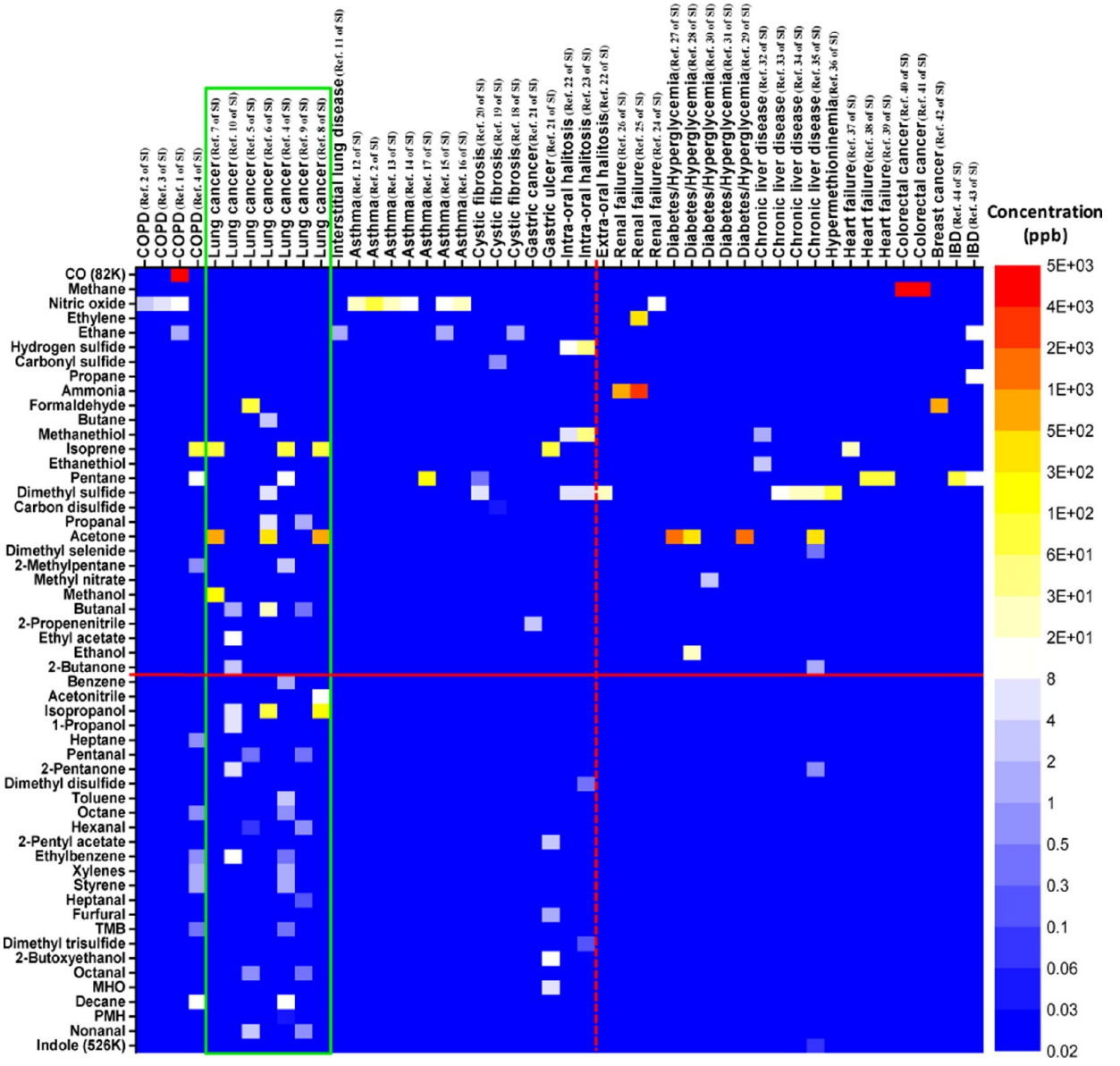

FIGURE 3. Concentration map of indicative breath compounds with clinical significance for different diseases, specifying breath concentrations (in ppb_v) averaged between the breath levels in diseased states and controls/treated-states. The diseases are ordered so that pulmonary and UGI diseases are listed left of the vertical red-dashed line. The compounds are listed in an increasing order of BPs (values in parentheses are the compound's BP in Kelvin) with the horizontal red line arbitrarily dividing the compounds between high (above) and low (below) volatilities. Abbreviated compounds are carbon monoxide (CO), 1,2,4-trimethylbenzene (TMB), 6-methyl-5-hepten-2-one (MHO), and 2,2,4,6,6-pentamethylheptane (PMH).

knowledge of the molecular identity of the targeted VOCs and their breath concentrations. Our survey of over 100 studies reporting on indicative exhaled VOCs and gases for 27 different clinical states disclosed only 44 different studies specifying the breath concentration of 54 VOCs in different combinations for 17 diseases (see Supporting Information (SI), Table S1). Hence, concise data on the breath concentration of breath markers is available for few diseases only. Interestingly, in 87% of the disease/VOC-concentration indications (119 in total), the marker levels were elevated in the breath of the diseased states compared to the levels in the controls or treated states (see SI, Table S1). Nevertheless, only 26% of the indicated disease/VOC combinations are cross-validated. Figure 3 shows a concentration map of the 54 clinically significant breath compounds for the above 17 diseases. These 17 diseases mainly fall into the following two groups: (i) diseases of the respiratory system and UGI track (see diseases left of the vertical dashed

red line in Figure 3) that involve the second path described in the previous paragraph (which enables bypassing mediation through the blood system); (ii) states involving the first path alongside accumulation of specific metabolites caused by the progression of the disease, for example, extra-oral halitosis, renal failure, diabetes/hyperglycemia, chronic liver disease, and hypermethioninemia.[1,2,6] Although more clinical studies are essential for producing a more accurate disease-VOC concentration map, initial efforts should focus on tailoring NMVSs for detecting the target compounds listed in Figure 3 and aiming for limits of detection (LoD) lower than the specified breath concentrations.

Unlike the diseases listed in Figure 3, only qualitative data exists on potential breath VOCs for many other diseases. In such cases, in order to provide a starting point for proof-of-concept sensing studies, estimations of VOC breath levels should be made based on the VOC's typical blood concentration (in vitro evaluated)[26] as well as its blood/air partition coefficient (λ_{ba}).[27] This approach is based on the notion that blood/air material exchange in the lungs is governed by vapor/liquid equilibrium. Consequently, the breath concentration of a VOC should be correlative to its blood concentration and strongly governed by the VOC's λ_{ba}.[3,27,28] In the absence of experimental λ_{ba} coefficients, estimated λ_{ba} values can be used based on either theoretical molecular descriptors or experimental physical properties (for example, water/air Henry's constants and rat-λ_{ba}).[3,28] On the other hand, the absence of VOC blood concentration data for a given disease makes it difficult to perform even rough estimations of the expected breath concentrations, even if λ_{ba} coefficients are available (see SI Figure S1). Nevertheless, according to Figure 3, the breath concentration does seem to be related to the compound's volatility or boiling point (BP) because low BP compounds (situated above the arbitrary horizontal red-dashed line) tend to appear in breath in higher concentrations than high BP VOCs. This might explain why there are more reports on the concentration of low BP compounds. This trend is most likely intensified by the common strategy applied in many clinical studies of "scanning" for indicative VOCs rather than searching for specific VOCs that are known to be biochemically related to the studied disease. Additionally, high BP VOCs were hardly reported for clinical conditions not involving the respiratory system or UGI tract (lower right quarter of the color map). This might reflect the absence of direct outgassing of VOCs into the airways through the second path described above. This absence in outgassing results in low expression of high BP VOCs in breath making it more difficult to detect them.

In the case of low BP VOCs at elevated concentrations up to a few ppm_v, for example, acetone and ammonia (see Figure 3), a wide choice of sensing platforms would be allowed (see Figure 4). In the case of uncertainty concerning the exact nature of the target VOCs or in the case of diverse print of volatile compounds (for example, see reported lung cancer breath markers bordered by a green rectangle in Figure 3), a semiselective sensing approach would be more feasible, since it does not require vigorous fabrication of a specific sensor for each marker. For example, an electronic nose system based on arrays of chemiresistive films of MCNPs or RN-CNTs could be used. This system has demonstrated potential as a breath testing platform for various diseases such as different cancers, multiple sclerosis, and chronic kidney disease.[13,14,29] To optimize the performance of such sensor arrays the diversity of the semiselective functionalizations of the different NMVSs should be increased. Because this approach relies on pattern recognition and machine learning algorithms, special care must be given to avoid overfitting the training data set, by, for example, limiting the number of sensors used in the array and increasing the training data set as large as possible.[5]

In case of high BP VOCs that are found in breath at low concentrations of single ppb_v and even lower, for example, indole (see Figure 3), their detection calls for the use of highly sensitive nanomaterial transducers, such as nanowire or nanotube based FETs. Additionally, highly specific recognition elements and appropriate surface treatments must be utilized so that specific receptor-analyte binding dominates over nonspecific interactions, and, therefore, reduce the chance of false positive detection (see Figure 4).[24] Therefore, these efforts must be accompanied by analytical evaluations of the differences between the characteristic breath compositions of healthy individuals versus individuals suffering from the disease. Such analytical assessments should be done using standardized chromatographic based techniques, for example, gas-chromatography mass-spectrometry (GC-MS) or proton transfer mass spectrometry (PTR-MS).[2,30,31] Because numerous research groups around the world are conducting such analytical studies, the identified target VOCs for each studied clinical state must be uploaded to a "Breath Cloud," a global breathomic[2] database, for enabling cross validation of the data and assisting collaborative research efforts around the world. Such a global effort has a high chance to succeed if the different analytical

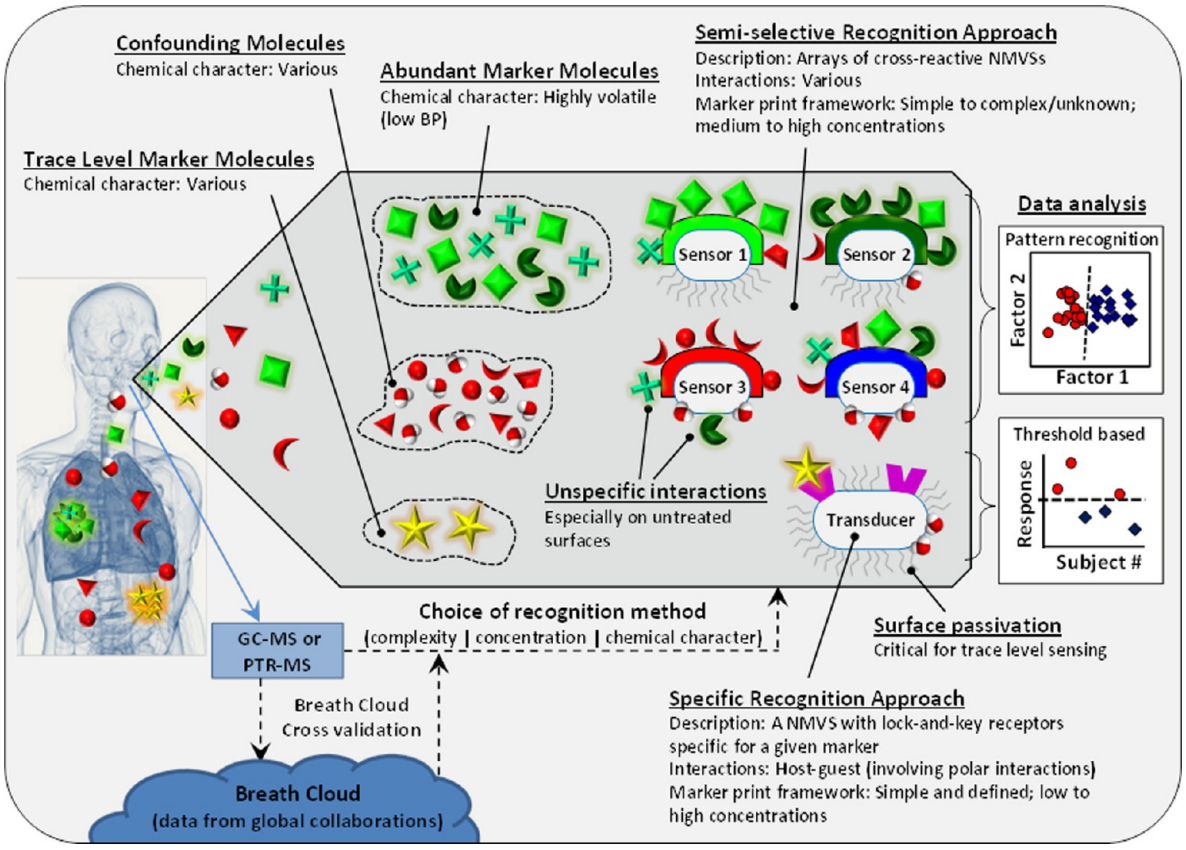

FIGURE 4. Schematic reviewing the links between the different frameworks of breath marker-prints and the appropriate sensing approach (specific vs cross-reactive approach).

procedures are standardized, an initial database is gathered from existing studies,[3] such as in Table S1 of the SI, and if strict criteria are set for uploading new information.

Additional considerations for fitting a sensing platform to a given disease include the physical and chemical characteristics of the different marker compounds. In this respect, VOCs are primarily divided into nonpolar and polar VOCs. The latter VOCs are generally easier to detect because they offer a wider range of possible molecular interactions, from strong H-bonding to weaker dispersion interactions.[6,21] Polar VOCs can be detected directly through receptor–analyte charge transfer, such as in the case of metal-oxide NMVSs,[6] or indirectly through receptor–analyte interactions that induce subsequent responses in the transducer, such as in the case of MCNP based chemiresistors. In addition, polar molecules can be accommodated through specific host–guest interactions making highly specific molecular recognition available.[12,21,22] In the case of nonpolar VOCs, the sensing mechanism must rely on indirect detection through dielectric changes and/or steric interactions resulting from weak dispersion forces between the recognition element and the molecule.[32] This condition implies that the geometry (shape and size) of the nonpolar VOC is an important factor for developing molecular recognition elements with enhanced selectivity. For instance, the use of cubic MCNPs as chemiresistive films (instead of spherical MCNPs) has been shown to enable discrimination on the basis of the chain length of straight alkanes.[33] On-chip amplifiers in the form of self-assembled polycyclic aromatic hydrocarbon (PAH) layers covering chemiresistive films of RN-CNTs provided the sensors with selective responses to polar and nonpolar VOCs and enabled the sensors to distinguish between a variety of VOCs in (constant) low as well as high humidity background.[11] The functionalization of Si-NW FETs by a monolayer of alkane-backbone silanes enhanced the selectivity of the sensors toward nonpolar VOCs (see Figure 2b) through what was shown to be an "indirect" steric molecular gating mechanism. In contrast, polar VOCs were detected more directly due to VOC-induced changes in the Si-NW charge carriers.[8]

Confounding Factors

The main technological challenge for sensors used for breath analysis is the requirement for trace-amount

detection of VOCs in the presence of real world confounding factors. Factors such as the chemical or physical instability of both breath samples and sensing elements as well as the variation of VOC background, humidity and temperature impose numerous problems for stable analysis of real-world breath samples.[20] Initially, the sampling process of exhaled breath and the delivery of the breath samples to the sensors can introduce considerable amounts of contaminants or lead to the loss of target VOCs. Apart from the previously discussed specific recognition approaches to minimize contaminant impact, these problems can be minimized by using proper sampling and preparation techniques that are integrated with the fluid delivery system of the gaseous sample to the sensor elements. Contrary to the commonly used technique of a storage container (for example, a collection bag) that often introduces contaminations and causes VOC loss following storage, the technique of "trapping" the VOCs on a sorbent material followed by thermal desorption (TD) is a promising approach.[1] This technique enables flexible preparation and long-term storage of the sampled breath through the use of a semiselective sorbent material that "traps" a spectrum of VOCs.[1,2] In case the TD process results in a reduction of the initial volume of the breath sample, the technique allows not only the reduction of the complexity of the sample but also substantial preconcentration of the sample. This approach has recently been implemented in the form of an on-chip microfabricated preconcentrator (μ-preconcentrator) which serves both as a VOC trap and a VOC injector into a subsequent analysis unit (see Figure 5a).[34] Nevertheless, special consideration should be given to the choice of sorbent material because of the risk of losing "information" in case some target VOCs do not adsorb well to the sorbent matrix.[1,34] This technique also offers easy integration with low volume fluid delivery and manipulation systems to further optimize the sensing capabilities of the NMVSs. For example, a μ-preconcentrator can be coupled to a microfabricated GC column (see Figure 5b) that ideally delivers each breath component separately to the NMVSs according to the compound's column-stationary-phase partition coefficient.[35] This setup adds the dimension of compound retention time to the sensing process and enables specific recognition of ppb_v VOC levels. An additional advantage of sorbent traps, particularly hydrophilic sorbents (for example, Tenax), is the possibility to perform significant dehumidification of the sample, which would considerably improve the performance of almost any NMVS. This advantage is especially important because a typical population of breath samples contains high (>25 000 ppm_v of water vapor at 25 °C) and variable levels of humidity that can significantly affect the NMVS sensing signals and screen the detection of target compounds.[20,25] To this end, humidity can also be successfully separated from other breath components using a multicapillary column (MCC), which is simply a bundle of over 1000 parallel microcapillaries that lowers flow resistance and therefore enables higher chromatographic flow rates of ~150 mL/min and isothermal separation of VOCs at ambient temperature.[36] Aside from incorporating dehumidification techniques that might risk loss of marker compounds, approaches of enhancing recognition element surface coverage[25] and algorithms for humidity calibration of the NMVSs (see Figure 5c) can be used to reduce the hindering effects of humidity.[20] However, such approaches alone can be limited if the responses of the NMVSs to VOCs and water molecules are not independent as a result of competitive adsorption/binding with the recognition elements. This limitation calls for the implementation of new recognition elements enabling high selectivity between different VOCs and water vapor.[10,21] Additionally, realistic sensing characterization should be carried out in respect to the VOC/humidity sensitivity ratios,[20] which should be evaluated at working humidity and VOC levels typical for the applied sensing setup.

The working temperature of different components of a breath testing system is also an important aspect that must be carefully considered. In the case of breath samples, the working temperature should not be too high so as to protect reactive breath compounds such as aldehydes from decomposition. This constraint limits the use of sensors based on metal-oxide nanostructures that operate better at high temperatures, unless the VOCs are extracted and transferred into an inert carrier gas or the sensors are locally heated. On the other hand, at low temperatures heavy VOCs will undergo condensation and polar VOCs might undergo dissolution in condensed humidity on inner walls of system components. In both cases, maintaining a stable temperature during the sampling, delivery and sensing steps is important; such control can be achieved by incorporating an on-chip embedded micro-hot-plate (see Figure 5d). Additionally, the exposure of NMVSs to continuous thermal cycles following multiple exposures to many breath samples might enhance aging mediated drift in the sensitivity of the sensors. This drift can be overcome by applying calibrations[20] or by achieving stable sensing layers through strong binding between the reception elements and the transducer to inhibit oxidation processes (see Figure 5e).[37] Alternatively, a long aging process could be useful in some cases for

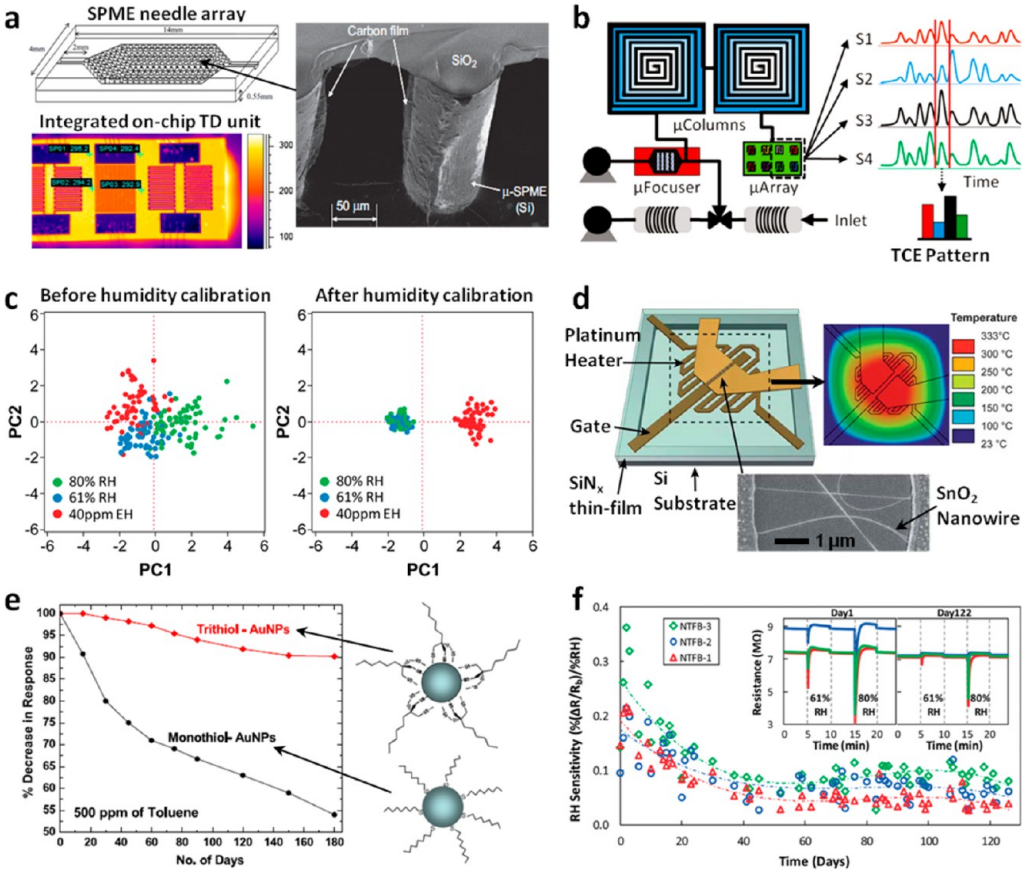

FIGURE 5. Means for tackling the implications of real-world confounding factors: (a) A μ-preconcentrator chip utilizes an array of solid-phase microextraction (SPME) needles coated with an in situ-grown carbon adsorbent film and integrated with an on-chip TD unit. Reprinted with permission from ref 34 with permission from Elsevier. (b) Schematic diagram showing the detection of trichloroethylene (TCE) within a complex sample using a micro-GC system consisting of a preconcentrating microfocuser (μF), two microcolumns and a microsensor array of Au-MCNP based chemiresistors. Reprinted with permission from ref 35. Copyright 2011 American Chemical Society. (c) Humidity calibration reduces humidity related response variations of an array of Au-NPs based chemiresistors exposed to clean moist air and air with 2-ethylhexanol (EH). Reprinted with permission from ref 20. Copyright 2012 American Chemical Society. (d) Temperature control of a SnO_2-nanowire based FET is achieved using an integrated micro-hot-plate. Reproduced from ref 38 with permission of The Royal Society of Chemistry. (e) Sensitivity stability of Au-NPs based chemirestors is improved by capping the NPs with trithiols instead of monothiols. Reproduced from ref 37 © IOP Publishing. All rights reserved. (f) Three Au-MCNPs based chemiresistors exhibit stable sensitivity toward water vapor following an aging period of ~40 days and almost identical response profiles after 122 days (inset). Reprinted with permission from ref 20. Copyright 2012 American Chemical Society.

achieving stable sensor operation over time (see Figure 5f).[20] Following these considerations, future breath testing systems will likely incorporate multidisciplinary approaches for reducing the different limiting factors related to breath testing alongside the use of nanomaterials tailored specifically to the detection of pre-evaluated target compounds.

Conclusion and Future Perspective

Successful deployment of chemosensors for breath testing would surely be cost-effective and highly beneficial for human health care, yet it involves many challenges. Discrimination between breath samples of diseased and healthy individual on the basis of trace level breath VOC differentiation demands high sensitivity and tunable selectivity. The demands are even further stiffened by real environmental conditions imposing unspecific interactions, humidity and temperature variations, and inhomogeneous test populations. Such a demanding framework compels an optimal choice of recognition approach and NMVSs for a given clinical state. For instance, in case clinical findings point toward a few specific breath markers with high BPs (indicating very low expression in breath), a specific recognition approach must be adopted employing highly sensitive nanomaterial transducers coupled to highly selective lock-and-key receptors. When the indicative breath print involves a complex and uncertain combination of VOCs, a semi-selective approach should be applied using arrays of cross-reactive NMVSs. Using this architecture relaxes the stressing

constraints on the NMVS's design resulting in a multipurpose device with low to medium levels of sensitivity toward the VOCs of interest. An array of NMVSs that combines both recognition approaches naturally performs an integration to yield a unique signal for complex but distinctive VOCs without requiring the mixture to be broken down into its individual components prior to, or during, the analysis.

Future accurate breath testing systems that aim to detect diseases should incorporate a combination of technologies that amplify the signals originating from the breath markers, but also decrease the parasitic response originating from different confounding factors. The concentration of the relevant breath VOCs should be increased and humidity levels decreased through the use of microfabricated sample preparation devices, for instance, using a μ-preconcentrator (see Figure 5a),[34] micro-GC (see Figure 5b)[35] or a MCC.[36] The processed sample will then be delivered to an array of NMVSs that will have an integrated on-chip temperature control unit (see Figure 5d). The type of recognition elements of the NMVS array would be tailored for maximum sensitivity and selectivity based on pre-evaluated breath markers. Following the trend of miniaturization in the world of technology, a breath testing system should eventually be able to fit into a casing as small as a smart-phone.

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Supporting Information. A list of indicative breath compounds for various diseases and blood-breath partition coefficient values of acetone and isoprene for healthy subjects. This material is available free of charge via the Internet at http://pubs.acs.org.

BIOGRAPHICAL INFORMATION

Gady Konvalina received his B.Sc. (cum laude) in Chemical Engineering from the Technion – Israel Institute of Technology (2007). Currently, he performs his Ph.D studies under the supervision of Prof. Hossam Haick.

Hossam Haick is professor in the Department of Chemical Engineering and the Russell Berrie Nanotechnology Institute since 2006. Prof. Haick's current research activities include nanoarray devices, noninvasive disease diagnosis, volatile biomarkers, and electronic charge transport through nanomaterials.

FOOTNOTES

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The authors declare no competing financial interest.

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