Breath and sweat analysis as a tool for medical diagnostics

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Abstract— A wide spectrum of volatile organic compounds (VOCs) is continuously produced in the human body and released through breath and sweat, thereby revealing important medical information. The variety of the analytical instrumentation used for measuring these biogenic VOCs is reviewed, highlighting the need to move towards field operability. The goal of this paper was to outline the potential of breath and sweat analysis for medical diagnostics and to introduce the idea of hand-held monitoring devices. Looking ahead, easy-to-use, low-power consumption, miniaturized instruments with processing capabilities and screening systems can further expand the use of breath and skin applications. Human breath and sweat present high potential as sources of volatiles for medical diagnosis and therapeutic monitoring. This medical knowledge should be integrated into personal care hand-held monitoring devices.

***Keywords*— expired air; skin; sweat; exhaled breath; Volatile organic conpounds (VOCs); biological fluids.**

I. INTRODUCTION

Breathing and sweating are two dynamic natural processes occurring in the human body resulting in the production of a wide spectrum of volatile organic compounds (VOCs). These are defined as carbon-based chemical molecules with a relatively high vapor pressure at room temperature. They can be categorized as *exogenous* VOCs, if they enter the human body originating from environmental exposure with subsequent exhalation (or as a drug which is metabolized to a volatile compound and exhaled) and as *endogenous* VOCs, if produced by the degradation of other biomolecules within human cells. This can be influenced by the consumption of foods or beverages or exertion of an effort. In addition, volatile compounds (such as hydrogen or methane) may be produced by bacteria in the gut or in the airways. The latter category is of great interest, as it may provide useful medical information reflecting internal bodily conditions. Other prominent examples are methyl-tertiary butyl ether (MTBE), a gasoline additive, and the resulting exhaled metabolite, tertiary butyl alcohol (TBA) which are used for exposure reconstruction [1,2]. Some examples of VOCs and their possible origin is given in Table 1.

Table 1: Examples of breath volatiles with endogenous and/or exogenous origin

Breath volatiles	Tentative origin
Acetone	Endogenous (lipolysis product)
Isoprene	Endogenous (mevalonate pathway)
Pentane	Endogenous (lipid peroxidation product)
Hydrogen, methane	Produced by bacteria in the gut, correlated with carbohydrate malabsorption
1,3-Butadiene, Acetonitrile, Furan, Furan, 2,5-dimethyl, Benzene	Exogenous (smoking)
2-Ethyl-hexanol	Exogenous (contaminant from tubing system)
3-Heptanone	Exogenous (metabolization of valproic acid, which is administered against seizures)
Hexanal	Endogenous (e.g. lipid oxidation) with higher concentration in breath of lung cancer patients
Sevoflurane	Exogenous (post-anesthesia)
Hexafluoroisopropanol (HFIP)	Exogenous (metabolite of the anesthetic sevoflurane) [3]
Chloroform	Exogenous
Trichloromethane	Exogenous (hot showers)
Tetrachloethane	Exogenous (dry cleaning)

Nevertheless, these few biogenic VOCs are considered a small part of the at least ~1750 VOCs emanating from the whole human body (also named as “volatilome”), as presented in Table 2. In addition to making use of endogenously produced volatiles, breath tests also rely on small amounts (possibly labelled) ingested compounds, observing their volatile metabolites (such as $^{13}CO_2$) in exhaled breath, and thereby measuring the phenotypic activity of enzymes.

Table 2: Overview of the volatilome of the human body [4]

Biological fluid/organ	Number of VOCs	Biological fluid/organ	Number of VOCs
Breath	872	Saliva	359
Skin emanations	532	Urine	279
Feces	381	Blood	154

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II. HUMAN BREATH

The use of human breath for medical diagnosis is directly linked with the "father of medicine", Hippocrates (460–370 BC, Kos, Greece), who noticed the difference in the breath of diseased individuals in comparison with healthy persons. The first "modern" approach to exhaled breath was developed in the late 18th century by Antoine Lavoisier, who discovered carbon dioxide and its production in the body of guinea pigs with subsequent exhalation. These findings of Lavoisier form the basis of capnography, which is the most common breath test ever devised. Another early compound discovered in breath was acetone; Wilhelm Petters in Prague discovered acetone in exhaled breath of a diabetic patient in 1857 [5] and Johannes Müller made the first good quantitative measurements of acetone in breath in 1898 [6]. In 1971, Linus Pauling used gas chromatographic techniques to demonstrate that many different compounds (not yet identified at the time) appear in exhaled breath and therewith confirmed Hippocrates' early observations, boosting the research and interest around human breath. A historical overview of breath analysis is presented in Table 3.

Table 3: The most important milestones of human breath history [7]

Milestone	Observation	Instrument
Hippocrates (460–370 BC, Kos, Greece)	Correlated smell of breath with illness	Smell of patient's breath; Hippocrates described *fetor oris and fetor hepaticus* in his treatise on breath aroma and disease
Antoine Lavoisier (1784)	Discovered CO_2 and its production in guinea pigs	An in-house apparatus
Nebelthau (1897)	Preliminary measurement of acetone in breath of diabetics	Colorimetric assay – diabetic's acetone change the colour of alkaline iodine solution
Francis E. Anstie (1874)	Isolated ethanol from breath (the first ethanol breath test)	Colorimetric assay - (breath alcohol turned the chromic acid solution from red-brown to green)
Linus Pauling (1971)	Human breath is a complex gas, containing over 200 different VOCs in picomolar concentrations	Gas–liquid partition chromatography analysis
> 1990s	Detection and identification of various medical diseases; early stage lung cancer, breast cancer, heart transplant rejection, tuberculosis, pseudomonas	Chromatographic and spectrometric methods with preconcentration enrichment step, optical techniques (e.g. laser absorption spectrometry, infrared spectroscopy), chemical sensors, sensors array (e-noses), etc.

Human breath mainly consists of water vapor, oxygen, nitrogen, inorganic gases (e.g. CO_2) and VOCs. The latter, although being a small fraction, are hundreds in number and associated with normal metabolism. Some of them are produced endogenously, while others are entering the human body by exposure to environmental VOCs. The origin of the majority of them is unknown. Food consumption and medication are important factors along with smoking. The majority of breath volatiles is appearing in the ppb_v to ppt_v (parts per billion per volume of air to parts per trillion per volume of air) concentration range. However, their concentration is changing in pathological conditions enabling their use as novel medical diagnostic tools for various types of cancers, oxidative stress, asthma, kidney failure and other medical disorders [8]. The most abundant VOCs in human breath are acetone (median concentration approximately 400 ppbv), isoprene (~100 ppbv), methanol (~150 ppbv) and ethanol (~100 ppbv). Acetone is formed from acetoacetate by acetoacetate-decarboxylase, whereas isoprene is a by-product of the mevalonate pathway and also produced in the periphery of the human body [9]. Breath sample collection is considered non-invasive and patient-friendly. It offers a unique monitoring window of blood volatiles (VOCs can be exchanged across the alveolar-blood capillary membrane and vice-versa), as well as, for the state of the lung. Next to multiple sampling, real-time measurements can be also performed enabling sampling during every human activity, even during sleeping. Breath is collected during exhalation into storage containers (inert Tedlar bags, canisters, glass tube) or using commercial breath samplers (e.g. BIO-VOC, Markes International, UK). The collected sample is analysed in the lab by trained personnel using hyphenated analytical techniques. The sample is thermally desorbed to a Thermal Desorption unit coupled to a Gas Chromatography (for mixture separation) and a Mass Spectrometry (for compound detection); TD-GC-MS. The low-concentration of breath volatiles is compromised though the use of a preconcetration step for sample enrichment. This is achieved through sorption trapping. Different adsorbing materials are used for trapping the various analytes; solid phase micro-extraction (SPME), solid phase extraction (SPE) with thermodesorption (TD) and needle trap devices (NTD). A variety of sorbent materials are available in the market based on the targeted chemical species; prior sampling the trapping material need to be regenerated for minimizing the presence of background volatiles. The main analytical technologies used for the analysis of breath volatiles [10] are given in Table 4. Current methods of analysis (with the exception of sensors technology) are laboratory based with limited portability for field settings. Emerging technologies enabling the development of promising hand-held analyzers are based on the latest advancements in microfabrication (e.g. development of micro GCs) and silicon micromaching technologies (e.g. preconcentrators, separation columns, detectors). A step towards this direction is the recent development of advanced sensors for breath sampling based on nanoscale technology; semiconducting DNA-carbon nanotubes [11] and specially coated gold nanoparticles [12]. Another quite promising trend is the use of chemical sensors in smart phones. In such an application, carbon nanotubes were integrated in an iPhone for chemical sensing of ammonia and chlorine for environmental applications [13]. "Smart

sensing" based on miniaturised sensing modules adapted in smart phones seems to be a feasible emerging technology with high potential.

Table 4: Main analytical instrumentation [10]

	Analytical Instrument	Advantages	Disadvantages
Hyphenated instrumentation	Gas chromatography -mass spectrometry (GC-MS)	High analytical identification power (through mass-spectra and retention time)	Bulky (but miniaturized GC instruments are in development) High-power consumption Cannot perform on-line or on-site measurements
	Proton transfer reaction-mass spectrometry (PTR-MS)	Real-time measurements	Bulky High-power consumption
	Selected ion flow tube-mass spectrometry (SIFT-MS)	Real-time measurements	Bulky High-power consumption
Ion mobility spectrometry (IMS)	Multi-capillary column-IMS (MCC-IMS)	Real-time measurements Transportable	Lack of library High-power consumption
	Aspiration-IMS	Real-time measurements, Hand-portable	Lack of library
	Differential-IMS (D-IMS, known also as field asymmetric-IMS, FAIMS)	Real-time measurements Miniaturized	Lack of library
Spectroscopy	Laser spectroscopy	Applicable mainly for small molecules (i.e. ethane, propane, methane, hydrogen, carbonyl sulfide or pentane) Real-time measurements	Bulky but with potential of miniaturization
	Infrared spectroscopy	Easily transportable instruments exist	Isotopic ratio $^{13}CO_2/^{12}CO_2$ for specific breath tests (e.g. the ^{13}C-uracil breath test, ^{13}C-dextromethorphan breath test, ^{13}C-pantoprazol breath test).
Sensors	Sensors or array of sensors (electronic noses)	On-line and on-site applications	Need for pattern recognition methods

A number of breath tests have become widely acceptable and have been approved by the US Food and Drug Administration (FDA) or the European Medicines Agency (EMA). In particular, the ^{13}C-urea breath test for gastric infection by *H. pylori* is the only FDA-approved breath test using ^{13}C-labeled precursor compounds. The most widely applicable breath tests are presented in Table 5.

Table 5: Approved breath tests [14]

Breath carbon dioxide (CO_2) test for capnography; Breath carbon monoxide (CO) test for neonatal jaundice (CO is produced by heme catabolism); Breath hydrogen and methane tests to detect disaccharidase absorption deficiency, gastrointestinal transit time, bacterial overgrowth, intestinal status; Breath nitric oxide (NO) test for monitoring of asthma therapy; Breath test for detection of heart transplant rejection; ^{13}C-urea breath test for detection of *H. pylori* infection; Breath ethanol (CH_3CH_2OH) test for blood alcohol (law enforcement)

Nowadays, another interesting line of research is that of correlating bacteria and fungi with breath volatiles. For example, in case of abnormalities in consuming lactose or fructose by gut bacteria (i.e. not adsorbed or metabolized due to malabsorption or due to enzyme deficiency e.g. lactase in fructose or lactose) hydrogen and methane are detected in breath. Particularly interesting are also compounds that are not normally observed in exhaled breath such as hydrogen cyanide (HCN) and ethyl-2-methylbutyrate produced by *Pseudomonas aeruginosa* or 3-phenylfuran produced by *Streptococcus pneumoniae*. Last but not least, is the extent of breath testing to animals for monitoring their daily health care [15].

III. HUMAN SWEAT

As mentioned earlier in Table 1, human sweat produces a variety of VOCs (~500 volatiles); it is almost as rich in volatiles as the human breath. The majority of the emitted compounds are hydrocarbons, acids, esters, alcohols and nitrogen containing compounds followed by furans and ethers, ketones, aldehydes, sulfur and halogen containing compounds. Skin emanations can contain useful information for medical diagnosis and therapeutic monitoring. Particularly interesting compounds are aldehydes and ketones, which are thought to be related to oxidative degradation and oxidative stress. Some VOCs are emitted both from human breath and skin such as acetone and ammonia. There is a great variety among the odors of individuals, which is further related to other factors such as the diet, the age, disease, etc. Other contributing factors are the wide use of personal care products such as the deodorants and perfumes. A number of studies were especially focus on skin emanations as attractors to mosquito [16]. A variety of analytical instruments have been employed for the determination of skin or sweat emanations; secondary electrospray ionization atmospheric pressure MS (SESI-API-MS), PTR-MS, SIFT-MS, MCC-IMS, SPME-GC-MS and thermally desorbed membranes using TD-GC-MS. In studies of skin measurements, certain parts of the human body are mainly targeted; axillae, hands and feet. The most frequently detected skin volatiles are acetic acid, propanoic acid, 3-hydroxy-2-butanone, hexanal, isovaleric acid, methyl hexanoate, 6-methyl-5-hepten-2-one, octanal, 2-ethyl-1-hexanol, limonene, benzyl alcohol, undecane, nonanal etc. [17]. The use of MCC-IMS for real-time measurements of skin volatiles revealed the detection of: 3-methyl-2-butenal, 6-methylhept-5-en-2-one, sec-butyl acetate, benzaldehyde, octanal, 2-ethylhexanol, nonanal and decanal [18]. Currently, skin cancer volatiles were detected using DNA-carbon

nanotube chemical sensor arrays, highlighting promising applications towards melanoma detection [19].

IV. HUMAN BREATH AND SWEAT

In confined spaces, humans emanate a variety of VOCs due to the physiological function of breath and skin processes. These volatiles are soon smelled by canines searching for helpless survivors under the rubble. In this content, experiments with human individuals were performed in confined spaces in an attempt to model the entrapment condition under the ruins of collapsed buildings due to earthquakes or terrorist attacks. In Table 6, confined space experiments are presented. In these simulated experiments, a number of VOCs was found to be able to travel through a model of the debris (a glass column containing cassettes of typical building materials). The experiments were performed after ethical approval with volunteers enclosed for some hours in a house-made environmental chamber [20,21] or using a commercial body plethysmography chamber [22].

Table 6: Experiments in confined spaces

Instrument	Volatiles	Origin	Reference
MCC-IMS	Carbon dioxide, ammonia, acetone and isoprene	Breath and sweat	[20]
MCC-IMS and TD-GC-MS	2-ethyl-1-hexanol, acetone, acetophenone, ammonia, benzaldehyde, benzene, 1-methylethyl, decanal, hexanal limonene, octanal, nonanal	Breath and sweat	[21]
Selective reagent ionization Time of Flight MS in NO^+ mode (SRI-TOF-MS-NO^+)	propanal, hexanal, heptanal, octanal, nonanal, 2-methyl 2-propenal, acetone, 2-butanone, 3-buten-2-one, 6-methyl-5-hepten-2-one, 2-methyl 2-pentene, DL-limonene	Breath	[22]

CONCLUSIONS

Nowadays, VOCs profiling has become an attractive diagnostic method for clinicians and researchers. Time has come for breath and sweat analysis to be transformed to novel hand-held portable diagnostic devices.

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