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Non-invasive investigation of local skin chemistry

KEYWORDS: *Volatile organic compounds; gas chromatography-mass spectrometry; pH; tissue dielectric constant*

ABSTRACT:
Local skin chemistry was investigated in human participants using non-invasive approaches to characterise volatile organic compounds, cutaneous pH and tissue dielectric constants (TDC). A total of 26 volatile compounds were identified through headspace solid-phase microextraction sampling with gas chromatography-mass spectrometry analysis. Acids and aldehydes were the predominant volatile species, accompanied by a variety of alcohols, ketones, hydrocarbons and esters. Measured values for pH and TDC were within the normal range for healthy skin. Principle component analysis classified participants into sub-groups, wherein specific volatile markers and skin pH were found to be strongly predictive of participant gender.

INTRODUCTION:

Human skin is a region of high metabolic activity where secretion of a rich variety of biomarkers occurs throughout the stratum corneum (SC). Skin-derived volatile organic compounds (VOCs) are attracting increasing scientific and clinical interest as a non-invasive route to probe the body's biochemistry for diverse applications including medical diagnostics,(1) forensics,(2) security(3) and the design of perfumes and deodorants.(4) Studying skin VOCs requires a high degree of analytical sensitivity. Solid-phase microextraction (SPME) with gas chromatography-mass spectrometry (GC-MS) has emerged as the method of choice in this regard, owing to the sensitivity and identification capabilities of MS coupled with the ease of performing headspace sampling via SPME.(5-6)

Our skin is a constant source of VOCs which contain the footprints of cellular activities. As a result, VOCs can also reveal important biochemical information about the skin itself, with emerging reports of characteristic volatile profiles for chronic wounds,(7) skin barrier impairment,(8) and melanoma.(9) Skin VOCs are derived from apocrine, eccrine and sebaceous gland secretions, and their interactions with the skin microbiota. This can lead to great topographical diversity in skin volatile emissions which is not well characterised to date. Elucidation of volatile compounds of importance for dermatological, diagnostic or other applications will be dependent on an in-depth understanding of healthy volatile profiles and their relation to the local skin chemistry. The aim of this work was to investigate local skin chemistry non-invasively in terms of volatile emission profiles, cutaneous pH and barrier function measured *via* tissue dielectric constants (TDC). These measurements are invaluable for determining the baseline features of skin chemistry for future comparison in research and applications in diagnostics, dermatology and product design.

MATERIALS AND METHODS

Method development

SPME fibres were initially screened for skin VOC sampling using 3 different adsorbent phases; 100 µm polydimethylsiloxane (PDMS), 85 µm polyacrylate and 50/30 µm divinylbenzene/carboxen-polydimethylsiloxane (DVB/CAR-PDMS) (Supelco Corp. USA). A wearable concentration platform(8) was assembled using a glass holder (3 mL internal volume, Pyrex, Fisher Scientific Ireland) and two septa (Supelco Thermogreen LB-2 Septa plug, Sigma Aldrich, Ireland) to suspend the SPME fibre in an enclosed area above the skin headspace. The glass housing was affixed to the volar forearm with surgical tape for sampling over 1 hr. Samples were desorbed for 2 min at 300 °C in the inlet of an Agilent 6850 Network GC-FID system in splitless mode. Separations were performed on a DB-5ms column (25 m x 0.25 mm x 0.25 µm) with hydrogen carrier gas (1.3 mL/min). The GC oven temperature was set to 30 °C for 1.65 min, followed by heating to 250 °C at 7.20 °C/min. The detector was maintained at 260 °C with 30 mL/min hydrogen and 340 mL/min air.

Participant recruitment

6 healthy participants (3 males, 3 females; aged 22-28) were recruited having given their informed consent. Ethical approval for skin volatile sampling was obtained from Dublin City University Research Ethics Committee, and the study was carried out according to the Declaration of Helsinki. Before samples were collected from skin, participants washed their hands and arms with tap water, followed by drying with paper towels to minimise contributions from exogenous compounds on the skin.

Skin volatile sampling and analysis

50/30 µm DVB/CAR-PDMS Stableflex (2 cm) assemblies were selected for skin volatile sampling within the wearable concentration platform described above. SPME extraction time profiles were investigated. 15 min was required to reach equilibrium and was the sampling duration employed herein. SPME samples were desorbed

within a SPME inlet liner in the injector of an Agilent 6890 GC connected to an Agilent 5973 mass selective detector at 250 °C for 2 min in splitless mode, with separation on an SLB-5ms column (30 m × 0.25 mm × 0.25 µm, Supelco, USA) with helium carrier gas (1 mL/min). The GC oven temperature was set to 40 °C for 5 min, after which the oven was programmed at a rate of 15 °C/min to 270 °C. The MS was operated at a scan rate of 3.94 s^{-1} and a range of 35-400 *m/z*. An ionizing energy of 70 eV was utilised, and the ion source temperature was maintained at 230 °C. Data were analysed using OpenChrom (Lablicate GmbH, Germany). Compound identification was performed using the NIST 2005 Library (match threshold >70%) and supported by retention index (RI) matching (±10 RI unit threshold) using a standard mixture of saturated alkanes (C_7-C_{30} Sigma Aldrich). Tabulated chromatographic peak areas of reliably identified compounds were imported into RStudio (version 1.1.456, R 3.5.1) for statistical analysis and generation of figures.

Tissue dielectric and pH measurements

Skin TDC was measured using a Delfin MoistureMeterD (Delfin Technologies, Finland) at an effective measuring depth of 0.5 mm. Skin pH measurements were performed using a Hanna HI 1413B flat tip pH electrode connected to a Hanna HI 2210 pH meter (Hanna Instruments Inc, Bedfordshire, UK). All measurements were performed in triplicate.

Results and Discussion

Comparison of SPME adsorbent phases revealed that PDMS-DVB/CAR yielded the greatest number of compound peaks (131, compared to 117 in PDMS and 118 in polyacrylate) as well as capturing compounds across a wide boiling point range as shown by the variety of peaks at all retention times in Figure 1. The effectiveness of PDMS-DVB/CAR agrees with manufacturer recommendations that indicate this fibre phase is ideal for capturing a wide variety of volatile compounds. The fibre coating includes PDMS which is ideal for sampling non-polar compounds, Carboxen is porous and polar which is useful for trapping low volatility compounds while the divinylbenzene phase has enhanced capacity for adsorbing polar analytes.

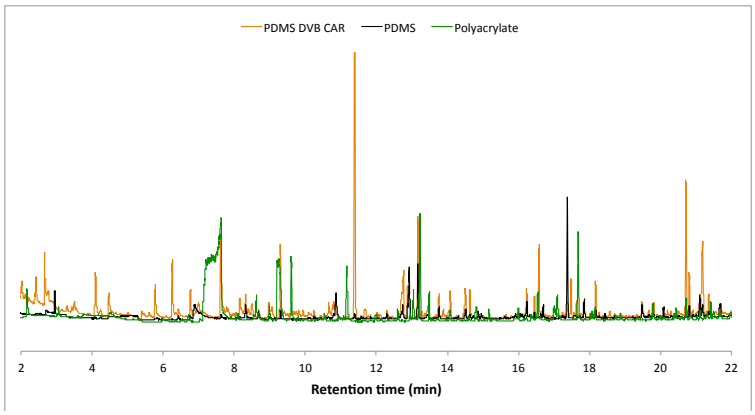

Figure 1. Overlaid chromatograms comparing 3 SPME fibre phases for sampling skin VOCs.

A variety of compound classes emanating from skin were identified from PDMS-DVB/CAR SPME pre-concentration followed by GC-MS analysis, including aldehydes, alcohols, acids, ketones, hydrocarbons and esters. A total of 26 compounds were identified in samples from the volar forearm (Table 1), 15 of which were common to all participants. Acids and aldehydes dominated the volatile profile, with hexadecanoic acid the predominant species, followed by tetradecanoic acid, nonanal and decanal. Several other frequently reported skin VOCs[5] were also observed in all participants including octanal, undecanal, and geranyl acetone. The predominant alcohol, 2-ethyl-1-hexanol, is often reported present in skin VOCs but has been linked to industrial origins. One ester (isopropyl palmitate) was also recovered in all samples and was attributed to exogenous sources.[10] Human skin is a constant source of VOCs as a result of glandular secretions and microbial activity, leading to characteristic individual profiles. For the primary constituents (acids and aldehydes), varying levels of fatty acids on the surface of the skin, and variation in the rate of their oxidative degradation can impact the volatile profile. Sebaceous secretions can also impact many VOCs emanating from skin, including hydrocarbons and ketones.[11-12]

Table 1. The 26 compounds identified in participants' skin headspace, shown with corresponding CAS numbers and frequency of occurrence in males and females.

Number	RT (min)	Compound	CAS	Male	Female
1	7.92	1-Nonene	124-11-8	3	1
2	8.05	Nonane	111-84-2	0	1
3	9.22	Benzaldehyde	100-52-7	3	3
4	9.78	Octanal	124-13-0	3	3
5	10.15	2-Ethyl-1-hexanol	104-76-7	3	3
6	10.29	Benzyl alcohol	100-51-6	3	3
7	10.72	1-Octanol	111-87-5	2	2

8	11.08	Nonanal	124-19-6	3	3
9	12.19	Decanal	112-31-2	3	3
10	12.77	2-Decenal	2497-25-8	0	1
11	12.83	Nonanoic acid	112-05-0	3	3
12	13.23	Undecanal*	112-44-7	3	3
13	13.66	n-Decanoic acid	334-48-5	3	2
14	14.16	Dodecanal	112-54-9	3	2
15	14.39	Geranyl acetone	3796-70-1	3	3
16	14.46	Undecanoic acid	112-37-8	2	1
17	14.81	α-isomethyl ionone	127-51-5	0	1
18	14.89	Pentadecane	629-62-9	0	1
19	15.21	Dodecanoic acid	143-07-7	3	3
20	16.93	Tetradecanoic acid	544-63-8	3	3
21	17.03	Octanoic acid, octyl ester	2306-88-9	0	1
22	17.64	Pentadecanoic acid	1002-84-2	3	3
23	18.18	9-Hexadecenoic acid*	2091-29-4	3	3
24	18.32	n-Hexadecanoic acid*	57-10-3	3	3
25	18.66	Isopropyl palmitate	142-91-6	3	3
26	19.63	2-Ethylhexyl 4-methoxycinnamate	5466-77-3	2	3

* Tentative identification, match >10 RI units

The skin surface has an acidic pH, often referred to as the 'acid mantle' which is an important determinant for the growth conditions of microbiota and for maintaining good skin condition by supporting formation of the lipid barrier and SC homeostasis.[13] Measured skin pH values were all acidic (Table 2) with an average pH of 4.9 for males and 5.5 for females. These values are within the reported range for normal human skin which can vary from an acidic pH of 4.0 to a neutral pH of 7.0.[13] Higher levels of SC hydration are considered characteristic of a healthy intact skin barrier. SC hydration can be determined non-invasively by measuring surface electrical properties. Changes in SC capacitance are expressed as TDC values. Measured values for TDC (Table 2) were within the normal range, where men typically exhibit a higher TDC than women.[14]

Table 2. Measured values for skin pH and TDC.

Participant	TDC	pH
M1	33.2 ± 2.9	4.76 ± 0.03
M2	29.8 ± 0.8	5.07 ± 0.03
M3	39.9 ± 1.7	4.72 ± 0.06
F1	29.0 ± 1.9	5.24 ± 0.10
F2	31.4 ± 0.9	5.78 ± 0.02
F3	27.2 ± 0.4	5.39 ± 0.03

Principle component analysis (PCA) was performed on the combined skin volatile, TDC and pH data for all participants. As seen in Figure 2, participants were classified into 2 sub-groups (male and female). Male participants were clustered closely together and are differentiated from females primarily by their higher levels of 1-nonene, geranyl acetone, dodecanal and isopropyl palmitate. Additionally, male participants displayed higher concentrations of volatile acids (n-decanoic, dodecanoic, tetradecanoic, n-hexadecanoic acids) compared to female participants. These higher levels of acid compounds correlate with the lower skin pH values measured for males, and pH is a powerful predictor of participant gender. Men were also distinguished from females by a higher TDC, but to a lesser extent than skin volatiles or skin pH. Female participants are distinguished from males on PC1 primarily by their significantly higher average skin pH (pH 5.5 compared to 4.9). One female participant (F1) had a unique skin volatile profile with evidence for nonane, 2-decenal, undecanoic acid, and octanoic acid octyl ester which were not present in the other females as seen in Table 1. Pentadecanoic acid was also present at 3x greater abundance in F1 compared to the other two females and the contributions of these compounds are captured by the PC2 loading highlighting the difference in this participant's skin chemistry.

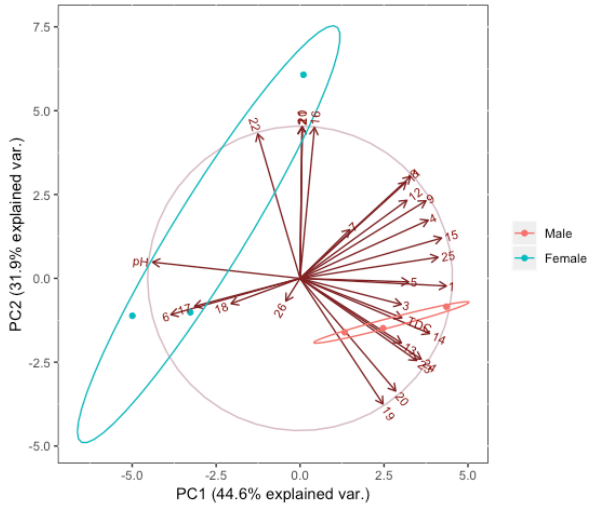

Figure 2. Scores plot from PCA of skin volatiles, pH and TDC. Compounds are numbered from 1 to 26 as shown in Table 1. Arrows (*i.e.* loadings) show the relative contribution of the variables to the scores plot.

CONCLUSION:

Local skin chemistry incorporating volatile metabolites, surface pH and TDC was characterised in 6 human participants. Male and female participants were clearly differentiated according to their skin chemistry, with males exhibiting lower levels of surface pH and higher levels of volatile acids, 1-nonene, geranyl acetone, and isopropyl palmitate. One female participant exhibited unique skin chemistry that differentiated her from the others. This was attributed to the presence of a number of volatiles including nonane and 2-decenal. This research is important for developing an understanding of the baseline features of skin chemistry and recruitment of a larger participant cohort for skin chemistry investigation is ongoing in our research group. Differences within a normal population need to be understood and controlled for before meaningful differences can be elucidated for applications in diagnostics, dermatology and fragranced product design. The present research presents a robust approach that will form the foundation of this future work.

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