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Applicability of emanating volatile organic compounds from various forensic specimens for individual differentiation

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ABSTRACT

Trace biological materials contain volatile profiles that have yet to be evaluated to determine their value in forensic investigations. The volatiles released by different biological specimens (hand odor, hair, fingernails and saliva) collected from twenty individuals were identified using a solid phase microextraction–gas chromatography–mass spectrometry method. The human scent compounds from each specimen, per subject, were evaluated using Spearman rank correlation to assess the applicability of these compounds for the differentiation of individuals. The volatile organic compounds from each specimen type were readily identified and discriminated. When conducting inter-subject discrimination within a single specimen type, greater than 98.9% of the samples, or individuals, were differentiated for all specimen types. When conducting inter-subject discrimination among the four specimen types 99.6% of the samples were differentiated, at the 0.9 correlation coefficient threshold. Additionally, the only occurrence of cross-correlation between specimen types was observed between hair and fingernails while there were no cross-correlations with hand odor or saliva thereby demonstrating the distinctiveness of these specimens.

1. Introduction

In 2008, over 4 million violent crimes were reported within the United States [1], which included rapes/sexual assaults, robberies, aggravated assaults and simple assaults. These types of crimes are often associated with physical aggression which consequently leads to the transfer of biological materials. Bodily materials such as fingernails, hair and saliva are often collected and subsequently analyzed by comparison matches, microscopic evaluations, and/or DNA analyses. In turn, this collected trace evidence may objectively link a suspect/victim to a crime and develop important investigative leads. Another form of trace evidence that is being collected more regularly by law enforcement agencies within the United States is human scent.

Human scent is defined as the most abundant volatile organic compounds (VOCs) that are identified in the headspace of a collected scent sample [2]. The chemical constituents of human odor have been shown to be qualitatively similar among individuals, however the quantitative abundances that they are produced make them characteristic to the individual they are derived from [2–5]. A combination of the presence and abundance of VOCs produces a chemical profile that is particular to an individual and therefore can be seen as a biometric measurement [3,4].

Many factors contribute to the composition of human scent, such as genetics, diet, environment, bacteria present on the body and exogenous materials. Internally derived human scent VOCs migrate to the outside of the body through secretions from three glands, the eccrine, apocrine and sebaceous glands [6]. Eccrine glands are sweat glands that are present all over the body with the greatest density being found on the palms of hands, soles of the feet and on the forehead [7]. With nearly 2–4 million eccrine sweat glands present in the body, an individual can secrete approximately 2–4 L of sweat per hour [8]. Eccrine secretions originate as a filtrate of blood plasma and are comprised of 99% water and 1% of other chemicals (e.g., electrolytes, metabolites and waste products) [7].

Another type of sweat gland, the apocrine gland, is found in the dermis and is associated with hair follicles. These sweat glands are only found in the axillae, perineal and genitals and the secretions of these glands are regulated by hormones. Apocrine secretions are basic [9] and include lipids, steroids and proteins [10]. The sebaceous gland, which is also present on hair follicles, secretes an oily, waxy substance, called sebum. Compounds such as free fatty acids (FFA), squalene, cholesterol, wax esters, cholesteryl esters and triglycerides, were among the types of compounds identified in sebum [11]. In contrast to the apocrine glands, sebaceous glands are present all over the body and the secretions from these glands

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function to coat the surface of hair and skin preventing them from becoming dry and brittle.

Previous research has been conducted with the intention of elucidating the identities of compounds being released from skin for cosmetic, medicinal and forensic applications [4,12,13]. Curran et al. [3,4] has shown solid phase microextraction–gas chromatography–mass spectrometry (SPME–GC–MS) as a viable route for the extraction and analysis of human scent volatiles collected from hands. The resultant human scent profiles were shown to be reproducible and unique to the individual. In one study, a population of 60 individuals was sampled revealing high, medium, and low frequency compounds that demonstrated a high degree of variability among the sampled population [5]. The reported compounds included a range of functionalities, such as alcohols, acids, aldehydes, alkanes, ketones, esters, and nitrogen containing compounds [2,5]. Through the use of statistical analysis, such as Spearman rank correlations, a high level of differentiation was obtained among the subjects and their corresponding chemical profiles [4,5].

Numerous researchers have explored the chemical constituents that are derived from hands [4,5,14–17]. In forensic science, hand scent is of particular interest to investigators since 73% of collected scent evidence in the United States is comprised of items which have come into contact with hands [18]. Attention is now being turned to other biological specimens, such as head hair, fingernail clippings and saliva, which can be collected non-invasively and could possess evidentiary value as scent evidence articles.

Head hair is a human biological specimen that is frequently deposited into the environment. Hair is constructed by a durable protein called keratin. The crystalline keratin is extruded from the hair follicle and emerges as a hair shaft resulting in the appearance of hair. The average rate of growth for hair is 1 cm/month but actual rates of growth can range from 0.6 to 3.36 cm/month [19]. Secretions from the sebaceous glands, which surround the hair follicle, and eccrine sweat glands, which cover the scalp, coat the hair and impart it with characteristic odors. The use of online supercritical fluid extraction gas chromatography–mass spectrometry, revealed compounds such as acids, cholesterols, esters and squalene [20] present in human hair extract.

Similar to hair, fingernails are also composed of keratin. The nail plate originates from the nail matrix (root) and rests on a vascular nail bed, which is seen through the nail plate. A mixture of soft keratin (similar to that found in skin) and hard keratin (similar to that found in the hair follicle) have been identified within human nail plates [21]. The average growth rate of fingernails is 0.1 mm a day; however, this rate can be impeded from aging and malnutrition. Much of what is known of the composition of fingernails pertains to its elemental composition and very little, if any, research has been conducted to assess the VOCs being released by this specimen.

Saliva, also, can be deposited during acts of physical aggression, such as biting or sucking. Salivary evidence has gained usefulness for identity and drug testing because of its ease of collection and availability, being that daily saliva production averages 1.5 L in volume. Saliva is produced from three major salivary glands, which include the parotid, submandibular and sublingual glands. The glandular contributions to unstimulated saliva include, in descending order, the submandibular with 65–70%, parotid with 20%, sublingual with 7–8% and the minor salivary glands with $<$10% [22,23]. During stimulated saliva production, the parotid gland increases its contribution to over 50% of the total secretion. The constituents of saliva include: water, proteins, fatty acids, amino acids, lipids, glucose, hormones, etc. [22]. The pH of saliva is nearly neutral (about 6 or 7); however, changes in the salivary flow can fluctuate the pH, such that a decrease in flow, results in a lower pH (5.3) while an increased salivary flow raises the pH (7.8) [23]. Chemicals present in the headspace of saliva samples have previously been explored, both forensically [24], for the identification of individuals, and medicinally [25], for the diagnosis of dental diseases.

The potential use of trace biological materials, such as hair, saliva, or fingernails, as scent sources for canine use has never been evaluated. Presently, there are various countries around the world, including the United States, that employ canines for human scent detection work due to their ability of identifying and discriminating human odor. Human scent, as a form of trace evidence, can provide the investigative team with a number of key elements in a criminal case, such as following a suspect directly from the scene of a crime, determining the direction of travel of the suspect, identifying the suspect following a scent line-up procedure, identifying a particular location by scent, or recover missing persons [26].

Aside from hand odor, the value of using these biological specimens as a source for individualization has yet to be determined. Furthermore, in order for these specimens to be employed in a canine detection setting, an instrumental evaluation of the discrimination power of these forms of trace evidence needs to be conducted, as well as assessing the variability of the detected chemical profiles. Although the authors recognize the importance of sampling a larger population, in addition to analyzing the stability of odor from various biological specimens, it was not the intent of this work to present such data. The purpose of this study was to conduct a preliminary assessment on the use of novel biological specimens for identification purposes. This study utilized solid phase microextraction–gas chromatography–mass spectrometry for the extraction and analysis of volatile organic compounds that are present in the headspace of biological specimens, such as hand odor, hair, saliva, and fingernails. Qualitative, semi-quantitative and statistical evaluations were performed with VOCs detected from biological samples to evaluate the applicability of these materials as viable sources for human scent discrimination.

2. Materials and methods

2.1. Materials

This study used methanol (HPLC grade, Fisher Scientific, Pittsburgh, PA) and ethanol (AAPER Alcohol and Chemical Co., Shelbyville, KY) for the pretreatment of the collection materials. Chemical standards for external calibrations were obtained from Sigma Aldrich (St Louis, MO) or Fisher Scientific (Pittsburgh, PA). The soap used to wash the hands and forearms was natural, clear olive oil soap (Life of the Party, North Brunswick, NJ). The fragrance free shampoo was from Jason Natural Products (Culver City, CA). The gauze pads were 100% cotton, sterile, 2 in. $\times$ 2 in., 8 ply, gauze sponges (DUKAL Corporation, Syosset, NY). The cotton swabs were 6″ in length, wood stem, sterile, cotton tipped applicators (Solon Manufacturing Company, Skowhegan, ME). Nitrile rubber combs were used for the removal of hair strands from the scalp (Krest Products Corp., Leominster, MA). Plastic manicure brushes were used to clean above and underneath the fingernails (YCC Products Inc., Placentia, CA) and chrome plated steel fingernail clippers were used for the collection of fingernails (Tweezerman International, Port Washington, NY). The glass vials used were 2-mL and 10-mL, clear, screw top with PTFE/Silicone septa (SUPELCO, Bellefonte, PA). The SPME fibers used were 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (CAR/DVB/PDMS; SUPELCO, Bellefonte, PA).

2.2. Pretreatment of the collection material

Prior human scent research, which utilized sorbent materials for the collection of hand odor, revealed that the collection materials, though sterile, initially possessed compounds found in human odor [2]. The pretreatment of cotton gauze for the collection of hand odor samples was conducted to ensure the removal of any background contaminants and entailed spiking the gauze pad with 1 mL of methanol and baking the gauze in an oven at 105 °C for an hour [16]. A pretreatment regime was also devised for cotton swabs used in the collection of saliva. Each swab was spiked with 250 μL of ethanol and placed in an oven at 105 °C for 1 h and then repeated. The analytical cleanliness of the cotton gauze and cotton swab was determined by SPME–GC–MS prior to sampling.

2.3. Sample preparation and collection of hand odor samples

The hand odor collection protocol used was originally published by Curran et al. [5] and is described here. Each subject washed their hands and forearms with olive oil soap for 30 s, rinsed under cool water for 2 min, air dried for 2 min, followed by rubbing the hands over the forearms for 5 min (in an effort to reconstitute the depleted secretions). The olive oil soap used to clean the hands of subjects, prior to scent collection, was selected because it possessed no human scent compounds, as determined by headspace sampling using SPME–GC–MS. Hand odor was collected from each subject by placing a pretreated 2″ × 2″ cotton gauze pad between the palms of their hands for 10 min. A modification to the original collection procedure was all hand odor samples were collected while the subject remained indoors. Once complete, the scented gauze pad was placed into a 10-mL glass vial and allowed 24 h to equilibrate, prior to SPME extraction. The entire procedure was repeated in triplicate for each individual sampled.

2.4. Sample preparation and collection of hair samples

All subjects were asked to discontinue the use of personal conditioners or hair care products and to wash their hair on the morning of sample collection with the fragrance free shampoo that was provided. The fragrance free shampoo used in this study was selected because it possessed the fewest number of human scent compounds, as determined by headspace sampling using SPME–GC–MS. The three human scent compounds identified from the shampoo were undecane, dodecane and tridecane. Undecane was not detected in hair from any of the individuals sampled. Dodecane and tridecane, which were detected from the hair of two and three subjects, respectively, were removed from scent profiles. Hair was collected from the subjects by running a comb through their hair and shaking loose strands onto a sheet of paper where the hairs were gathered and cut into smaller pieces with stainless steel scissors. Three hair samples, with an approximate mass of 10 mg, were collected from each subject (~30 mg total). Each hair sample was then transferred into a 2 mL glass vial and allowed 24 h to equilibrate, prior to SPME extraction.

2.5. Sample preparation and collection of fingernail clippings

Prior to sample collection, each subject washed their hands with olive oil soap to remove any debris from underneath their fingernails. Subjects lathered their hands with soap for 30 s, rinsed under cool water for 2 min and air dried for 5 min. As part of the rinsing process, a nail brush was given to each subject for further elimination of any possible remnants of debris under each individual nail. Each subject sampled themselves by clipping their own nails with stainless steel fingernail clippers. Three different fingernails from the same hand were collected and each fingernail clipping was placed into a 2 mL glass vial and allowed to equilibrate for 24 h, prior to SPME extraction.

2.6. Sample preparation and collection of saliva samples

Prior to the collection of saliva the subject was asked to discontinue eating, drinking and smoking 3 h prior to sampling. Subjects rinsed their mouth with tap water for a period of 1 min prior to immediate sampling. Each subject sampled themselves by swabbing each cheek for 30 s, to produce a homogenous sample, while wearing nitrile gloves. The swab was then placed into a 10-mL glass vial and was given 24 h to equilibrate, prior to SPME extraction. This procedure was repeated consecutively in triplicate.

2.7. SPME–GC/MS method

Volatile extraction from the vials containing the biological specimen was performed at room temperature for a period of 21 h using 50/30 μm DVB/CAR/PDMS fibers. The separation and analysis of the extracted volatiles was performed using an Agilent 6890 GC/5973 MS fitted with a 0.25 mM × 30 m HP5-MS column which had a phase film thickness of 0.25 μm. Helium carrier gas was held at a flow rate of 1.0 mL/min. The injection port was maintained at a temperature of 250 °C. The GC temperature program started at an initial temperature of 40 °C which was held for 5 min, followed by an increase of temperature at 10 °C/min to a final temperature of 260 °C which was held for 2 min, for a total run time of 29 min. The mass spectrometer transfer line was maintained at 280 °C and the source

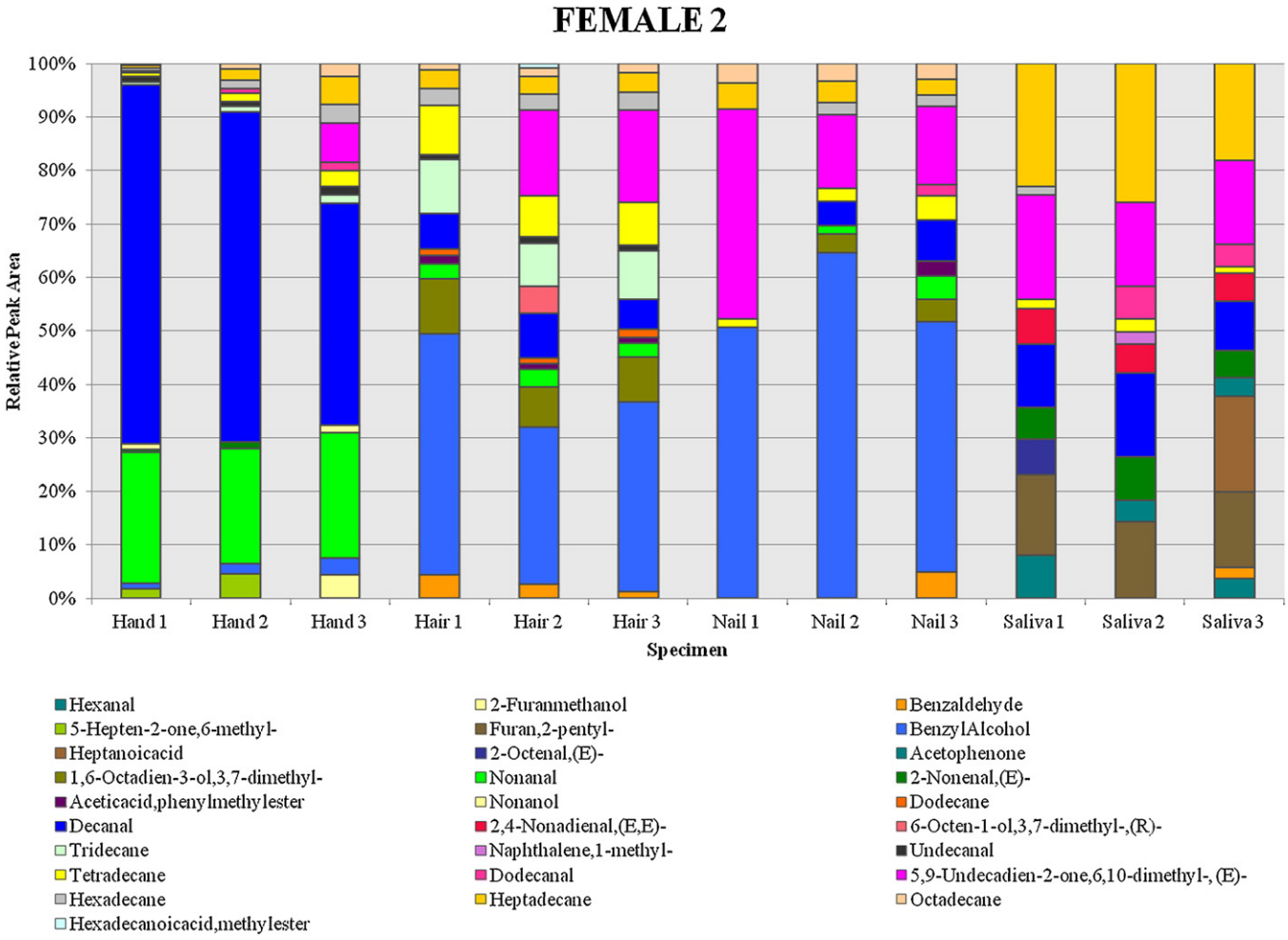

Fig. 1. Profiles of VOCs from biological specimens collected from a female subject.

temperature was 230 °C. Mass spectra were repeatedly scanned from 45 to 550 amu. Compounds were identified initially using the NIST 98 mass spectral reference library and were confirmed using a chemical standard.

2.8. Statistical analysis of biological specimens

Each biological specimen yielded an assortment of human scent compounds at varying abundances, for each individual, thereby producing a large amount of data. Therefore, principal component analysis (PCA), a multivariate data reducing technique, was used to analyze the volatile profiles of the biological specimens collected from an individual. Principal components are linear combinations of the experimental variables, such that the first principal component reflects the greatest amount of variation in the data set and the second principal component reflects the succeeding greatest amount of variation and so on [27]. When plotting the principal components, then patterns within the data set can be visualized.

Additionally, the averaged profile of VOCs for each biological specimen, per person, was ranked in ascending order using the data analysis technique of Spearman rank correlation. These ranks were used to correlate the scent profile of every collected sample against the other samples. Correlation among samples was expressed by a correlation coefficient, r_s, ranging in value from −1 to 1, demonstrating a negative or positive correlation, respectively [27]. Once all of the Spearman rank correlations were determined, assessments were made on the occurrence of matching errors (indistinguishable pairs) that can be assumed from the correlations coefficients of samples. The occurrence of matching errors for each specimen type at three correlation coefficient thresholds, 0.9, 0.8 and 0.7, were tabulated and used to assess compositional differences and/or similarities between individuals for each specimen type. Spearman rank correlation was selected for this work because it has been frequently used for the discrimination of hand odor samples [4,5,16,17] as well as for the discrimination of glass [28,29].

3. Results and discussion

3.1. Preliminary assessment

Prior to analyzing samples from the entire population, biological specimens collected from a three female and three male subjects were investigated to initially assess the VOCs being released by these specimens. The preliminary assessment was used to determine whether the scent from the different specimens was similar or different for each type, prior to analyzing samples from the remainder of the population. Only VOCs that have been previously cited in literature as originating from human specimens were used in the analysis of these samples.

The average mass amount of extracted VOCs for each specimen type was (in descending order) saliva with 177 ng (±68), hand odor with 94 ng (±63), hair with 33 ng (±37) and fingernails with 14.4 ng (±11). Color charts (as seen in Figs. 1 and 2) facilitate the visualization of the profile of human scent VOCs extracted from each sample. Each color in the color chart corresponds to a specific VOC and the length of the color bar corresponds to its relative abundance (as a percent of the total composition) within the sample. Easily seen in the color chart was both the reproducibility of the chemical profiles produced by the three samples for each specimen type (intra-specimen), as well as the differences in the chemical profiles between the specimen types (inter-specimen).

In addition to performing a visual inspection of the profiles of VOCs, a statistical technique, principal component analysis (PCA), was applied. Principal component analysis was used to identify groupings of the samples. First, the triplicate samples of each biological specimen collected from the male subject (intra-subject) were evaluated. When the first three principal components were plotted then four clusters, one for each specimen, could be visualized (Fig. 3). This further supports that the chemicals being released by each specimen type are characteristic of that specimen.

Subsequently, biological specimens from three females and three males (inter-subject) were also evaluated using principal

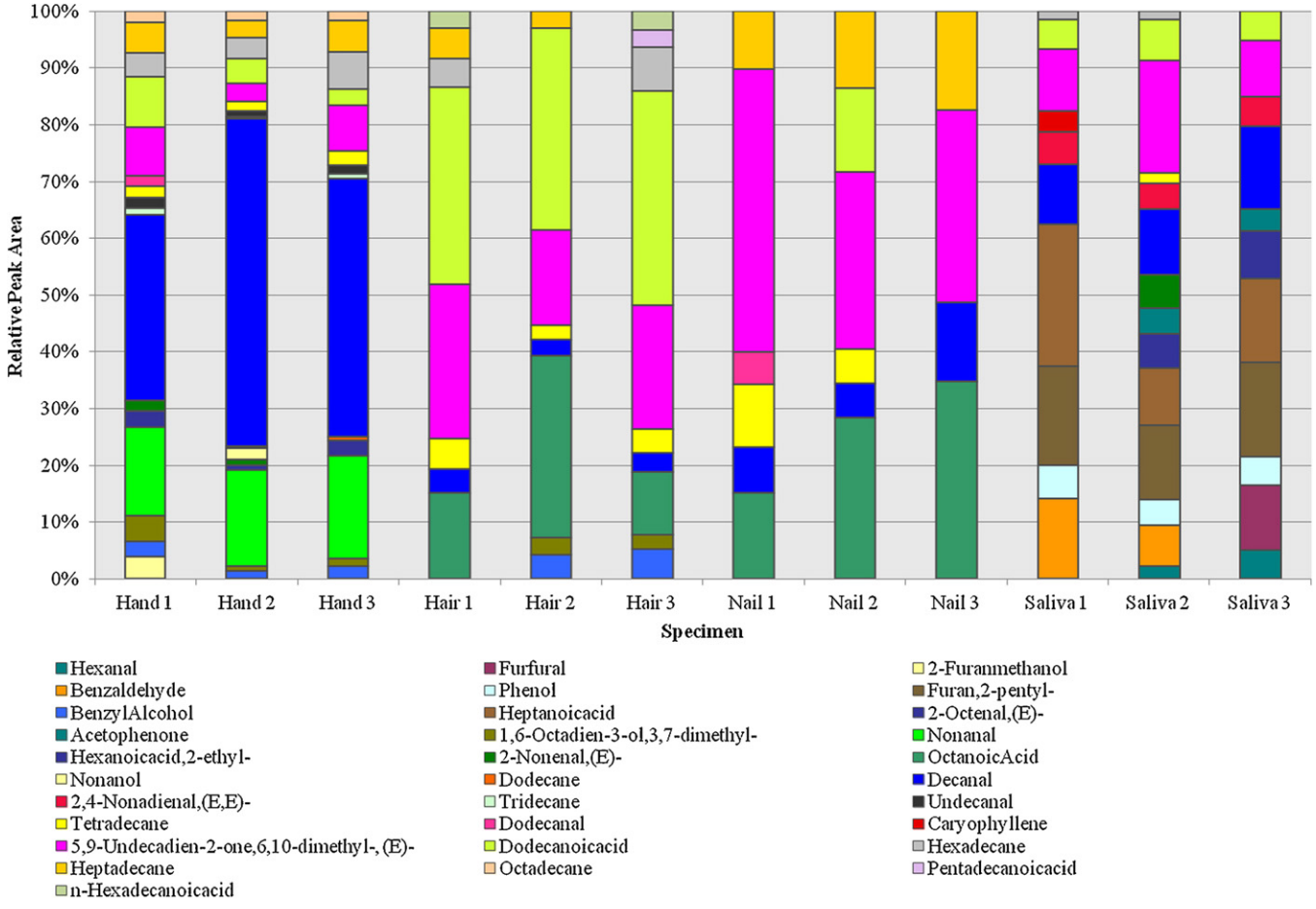

Fig. 2. Profile of VOCs from biological specimens collected from a male subject.

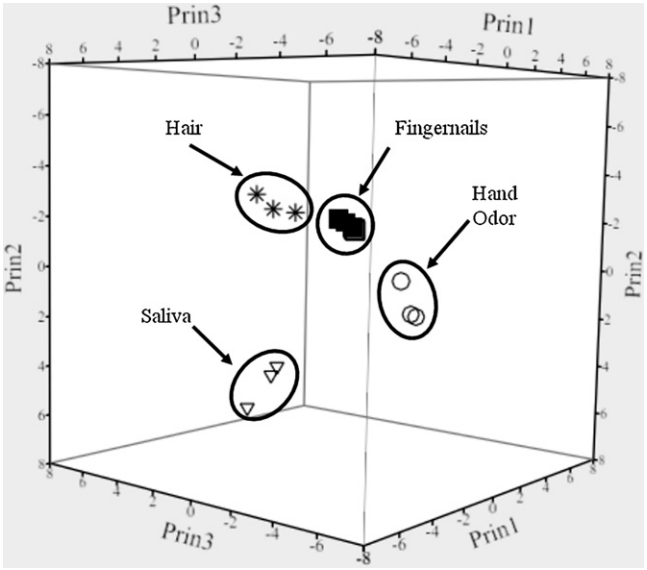

Fig. 3. PCA plot of biological specimens from Male 3 (cumulative variation 75.5%).

component analysis. For this portion of analysis, the triplicate samples of each specimen type from each individual were averaged. When plotting the first three principal components, three separate clusters can be seen for hand odor, saliva and hair/fingernails (Fig. 4). Principal component analysis revealed that hand odor and saliva are truly distinct; however, hair and fingernails have some similarities as depicted by their two clusters overlapping.

This assessment showed that saliva and hand odor released a different profile of VOCs that was specific to their specimen type, while also releasing the greatest and second greatest mass of VOCs, respectively. Hair and fingernails possessed some similarities in the presence of compounds, as seen with the overlapping clusters of hair and fingernail samples during the inter-subject evaluation (Fig. 4). Following the results of this preliminary assessment, it was determined that any comparisons made between individuals would be performed within a specimen type and not between types.

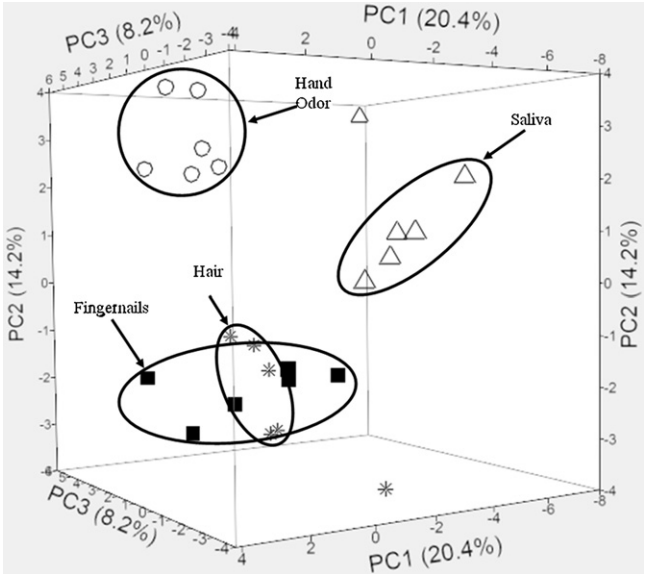

Fig. 4. PCA plot of biological specimens from three females and three males.

3.2. Volatile organic compounds detected among the biological specimens

Three samples of hand odor, hair, fingernails and saliva were collected from twenty subjects for a total of 12 samples per subject. The VOCs being released by each specimen were extracted and analyzed using headspace solid phase microextraction–gas chromatography–mass spectrometry (HS-SPME–GC–MS). The tentative identities of the extracted VOCs (when compared to the NIST 98 mass spectral library) produced a long list of compounds that ranged in functionalities which included acids, alcohols, aldehydes, aliphatic hydrocarbons, aromatic hydrocarbons, esters, heterocyclics, and ketones. However, a menu of compounds, which have been previously published in literature as being found in specimens collected from humans [2,4,5,12,14–17,24,30–39], were confirmed with certified reference standards and were subsequently used for data analysis. A total of 42 human scent VOCs were used to evaluate the inter-subject differences within each of the four biological specimens and the names and frequency they were detected from the twenty individuals sampled can be seen in Table 1. Additionally, the functional group distribution of VOCs from each biological specimen, which is represented as a pie chart, can be seen in Fig. 5.

Twenty-four human scent VOCs were detected from hand odor samples, twenty-three VOCs were detected from hair samples, eighteen VOCs were detected from fingernail samples and twenty-one VOCs were detected from saliva samples. Hydrocarbons contributed the most to the chemical composition of hand odor (29%), hair (31%) and fingernails (28%), while aldehydes contributed the most to saliva. Plenty of aldehydes were detected from the remaining three specimens, such that five aldehydes were identified from hand odor and hair, and four were identified from fingernails. Hair released the greatest amount of acids (17%) which was expected given that the sebum, secreted by sebaceous glands in the hair follicle, has been documented to contain saturated acids [32,40]. Alcohols provided the second most prevalent functional group for fingernails (22%), along with aldehydes, and third most prevalent functional group for hand odor (12%) and hair (17%). Lastly, the distribution of esters, ketones and heterocyclic compounds was less than 13% for all specimen types.

When assessing the VOCs detected from all of the specimen types, 17% of the human scent compounds detected were seen across all four types. However, there were greater similarities between hand odor, hair and fingernails while saliva appeared to be the most distinct. Four human scent compounds (3,7-dimethyl-1,6-octadien-3-ol, benzyl alcohol, nonanal, and octadecane) were detected in hand odor, hair and fingernails that were not detected in saliva. It is difficult to determine the exact source of these common VOCs because eccrine sweat glands contribute the most to hand odor, sebaceous glands contribute the most to hair odor and no glandular secretions contribute to fingernail odor; however, one similarity among these specimens is that skin (palms of hands), hair and fingernails all contain the keratin protein. Ultimately, the origin of these shared chemicals could not be identified. Additionally, there were 10 compounds (heptanoic acid, phenol, E,E-2,4-nonadienal, E-2-octenal, hexanal, caryophyllene, 1-methyl-naphthalene, 2-pentyl-furan, furfural and acetophenone) that were present in saliva were not detected in the other three specimens. Finally, two other biological specimen types, also, had VOCs that were specific to them such that hand odor had eight and hair had three compounds that were not detected in any of the other types.

Many researchers have evaluated the volatile components released by hands using SPME–GC–MS [2,4,5,12,15–17,31,41]; therefore, the subsequent three sections will take a closer look at

Table 1
Human scent compounds identified from each biological specimen and frequency of occurrence in collected samples.

Functionality	Compound name	Hand odor	Hair	Fingernails	Saliva
Acid	Dodecanoic acid	20%	30%	15%	60%
	Heptanoic acid				75%
	2-ethyl-Hexanoic acid	45%			
	n-Hexadecanoic acid		5%		
	Octanoic acid		10%	40%	75%
	Pentadecanoic acid		5%		
Alcohol	3,7-dimethyl-1,6-Octadien-3-ol	25%	65%	55%	
	2-ethyl-1-Hexanol		5%	15%	
	(R)-3,7-dimethyl-6-Octen-1-ol		25%	10%	
	Benzyl alcohol	15%	20%	65%	
	Nonanol	70%			
	Phenol				65%
Aldehyde	(E,E)-2,4-Nonadienal				40%
	(E)-2-Nonenal	50%			70%
	(E)-2-Octenal				55%
	Benzaldehyde		5%	5%	75%
	Decanal	100%	95%	90%	95%
	Dodecanal	70%	10%	10%	30%
	Hexanal				55%
	Nonanal	100%	55%	45%	
	Undecanal	100%	25%		
Hydrocarbon	Caryophyllene				60%
	Dodecane	25%	10%		
	Heptadecane	100%	75%	80%	35%
	Hexadecane	100%	80%	70%	55%
	Octadecane	85%	30%	30%	
	Pentadecane		5%		
	Tetradecane	100%	85%	70%	80%
	Tridecane	75%	25%	10%	
	Undecane	40%			
	1-methyl-Naphthalene				80%
Ester	Acetic acid, phenyl methyl ester	10%	35%	30%	
	Hexadecanoic acid, methyl ester		5%	5%	5%
	Nonanoic acid, methyl ester	10%			
	Octanoic acid, methyl ester	5%			
Heterocyclic	2-Furanmethanol	15%			
	2-pentyl-Furan				100%
	Furfural				5%
Ketone	2-Decanone	20%			
	(E)-6,10-dimethyl-5,9-Undecadien-2-one	100%	90%	80%	100%
	6-methyl-5-Hepten-2-one	30%			
	Acetophenone				25%

the volatile compounds extracted from hair, fingernails and saliva using solid phase microextraction.

3.2.1. Hair

The analysis of 60 collected hair samples revealed a range of compounds from various functional groups: acids, alcohols, aldehydes, esters, hydrocarbons and ketones. No volatile organic compound was detected from 100% of subjects sampled; however, the most prevalent compound was decanal which was detected from 95% of subjects. Two additional aldehydes (nonanal, and benzaldehyde) and a ketone (6,10-dimethyl-5,9-undecadien-2-one) were also identified in the headspace of hair samples, which have not been previously mentioned in literature. Though previously identified from human hair, squalene, cholesterol and nitrogen-containing compounds [40,42] were not detected in any of the hair samples with the HS-SPME–GC–MS method used for this study.

Originally, eleven different acids were tentatively identified (dodecanoic acid, nonanoic acid, octanoic acid, hexanoic acid, tetradecanoic acid, n-hexadecanoic acid, acetic acid, n-decanoic acid, pentadecanoic acid, pentanoic acid, and tridecanoic acid) from 17 of the 60 collected hair samples and 94% of the hair samples ($n = 16$) possessing acids were obtained from male subjects. However, due to poor instrumental compatibility, only the identities of four acids could be confirmed and therefore used during data analysis.

The higher occurrence of acids from the hair of male subjects was likely to be a result of the length of collected hair. Lipid-rich sebum is constantly being deposited onto the surface of the hair. As hair continues to grow, these lipids are being removed from hair through washing, exposure to UV light and contact with harsh chemicals (e.g., chlorinated pool water, hair dyes) [43,44]. The grooming habits of males usually includes regular haircuts, which for this study resulted in the collection of smaller pieces of hair (4.6 cm $\pm$ 4.1), in comparison to females (27.8 cm $\pm$ 7.5). Therefore, the samples collected from males primarily consist of hair that has been recently coated with sebum, therefore possessing a higher amount of lipids than females.

3.2.2. Fingernails

Until now, no study has explored the volatile organic compounds originating from fingernails. The functional groups of volatiles extracted from the headspace of fingernails were acids, aldehydes, alcohols, esters, hydrocarbons, and a ketone. Fingernails produced the least number of compounds ($n = 18$) than the other specimens. The most frequently observed VOCs from

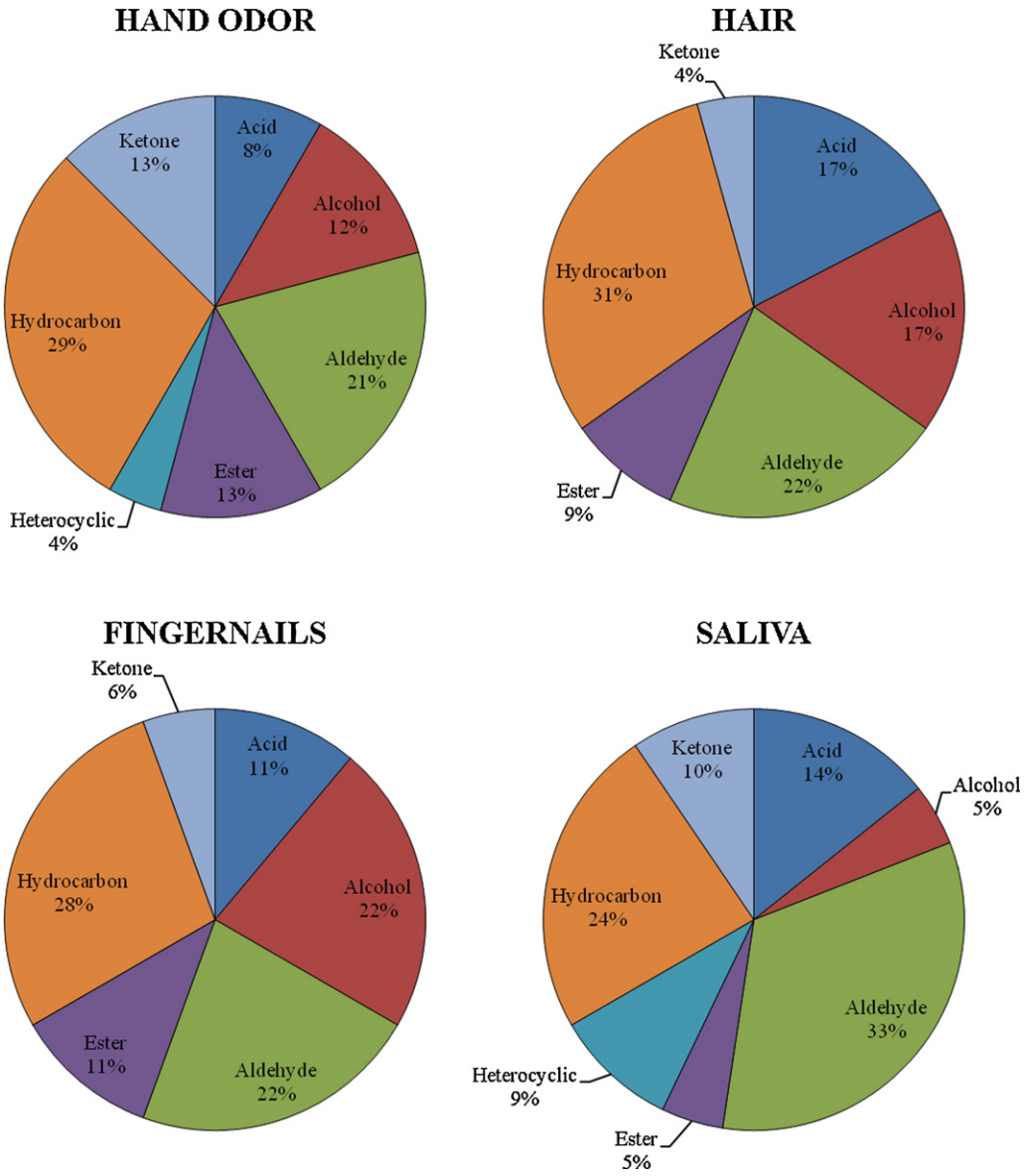

Fig. 5. Functional group distribution across biological specimens.

fingernails were decanal, heptadecane, hexadecane and tetradecance which were detected from 90%, 80%, 70% and 70% of subjects sampled, respectively. The functionalities of the VOCs extracted from fingernails were most comparable to those extracted from hair. There were five compounds that were identified in fingernails that were also present in hand odor and hair yet absent from saliva.

3.2.3. Saliva

Headspace solid phase microextraction of saliva samples revealed volatile organic compounds pertaining to functional groups previously identified in literature, as well as functional groups not previously mentioned. The functionalities of the compounds extracted from saliva included acids, alcohols, aldehydes, an ester, heterocyclics, hydrocarbons and ketones; however, SPME was unable to extract sulfur-containing compounds, nitrogen-containing compounds, phthalates, squalene or cholesterol, which have been previously identified from saliva [45].

Significant contributions to the chemical composition of saliva were from acids. Salivary pH normally ranges from 6 to 7, depending on the salivary flow [23], making saliva slightly acidic. Additionally, sources such as acids released by the salivary glands, bacteria, foods and beverages can contribute to the lowering of the pH in the mouth [45]. Notably, hexanoic acid was identified in 100% of the collected samples, which has been previously identified from human skin [14,16,31]; however, as it was explained previously poor resolution of the acid led to its removal from data analysis. 2-Pentyl-furan, a heterocyclic compound previously identified from saliva collected onto buccal swabs [41], was also detected at a high frequency with an occurrence of 100% from the twenty individuals sampled.

3.3. Spearman rank correlations within each specimen type

The profiles of human scent VOCs from each biological specimen were evaluated statistically to determine whether they were different for each of the twenty individuals sampled, thereby assessing the discrimination capabilities of each biological specimen. The replicate profiles of VOCs for each specimen type were averaged to produce a single profile of VOCs for all subjects sampled. Each subject's human scent profile was correlated, in a pair wise manner, to the remaining subjects using Spearman rank

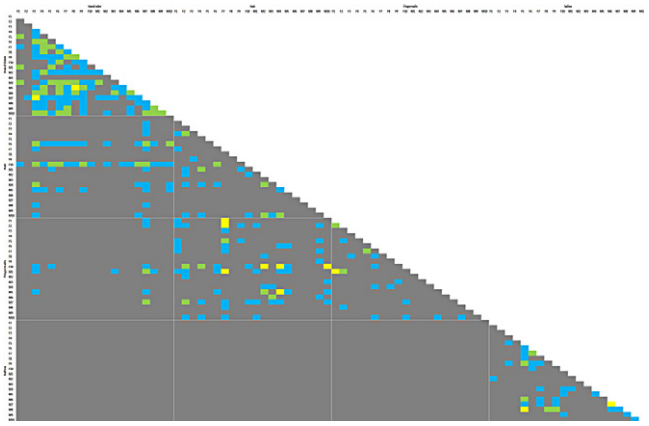

Fig. 6. Color map of indistinguishable pairs at the correlation coefficient thresholds of 0.9 (yellow), 0.8 (green) and 0.7 (blue) for all collected samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

correlations. The association between two people was represented by a correlation coefficient that ranged in value from −1 to 1, representing either a negative correlation or a positive correlation, respectively. The within-specimen comparisons of an averaged sample from twenty individuals provided a total of 190 possible pairs between the samples. For this work, the samples that produced a correlation coefficient of 0.7 and greater were assessed. A color map (also known as a heat map; Fig. 6) was used to represent the samples that correlated at >0.9, >0.8 and >0.7, which were denoted by a yellow square, blue square and green square, respectively. All of the samples that correlated at <0.7 were shaded in gray.

The profiles of VOCs for hand odor and saliva had the highest number of indistinguishable pairs (matching errors) at the 0.7 coefficient threshold and above. There were far fewer indistinguishable pairs for the hair and fingernail samples. A trend seen with all of the biological specimens was that the lowest number of indistinguishable pairs occurred at the 0.9 correlation threshold and increased as the threshold was lowered to 0.8 and then 0.7. Most analysts utilize the criterion that two samples must exhibit a high degree of similarity to be considered indistinguishable, which can be demonstrated by a high correlation coefficient (i.e., >0.9) when using a statistical technique such as Spearman rank. When applying that same criterion to this work, the number of matching errors was the lowest at the 0.9 threshold and discrimination of human scent can be achieved.

Once the number of indistinguishable pairs (matching errors) was tabulated then the percent of distinguished samples for each biological specimen was calculated (Table 2). At the 0.9 correlation threshold, both hand odor and saliva had the largest number of indistinguishable pairs ($n = 2$), followed by fingernails with one, and hair with no indistinguishable pairs. Therefore, the percentage of distinguishable samples for the four biological specimen types was above 98.9%, reaching as high as 100% for hair samples (seen in Table 2). These values demonstrate that though there are qualitative similarities within a specimen type there are also significant quantitative differences allowing for a high percentage of discrimination between individuals. These results imply that the VOCs released from biological specimens are specific to each individual and can be used for differentiation purposes with a low occurrence of matching errors (at a 0.9 correlation coefficient threshold).

Table 2
Percent of discriminated samples for each biological specimen.

Specimen	Correlation threshold		
	0.90	0.80	0.70
Hand odor	98.9%	79.5%	45.3%
Hair	100.0%	96.8%	88.9%
Fingernails	99.5%	97.9%	91.1%
Saliva	98.9%	96.3%	83.7%

3.4. Cross-correlation comparison of all collected samples

Spearman rank coefficient comparisons were conducted for all of the collected samples regardless of specimen type, so that the occurrence of cross-matching between specimen types could be assessed. The collected samples from all twenty individuals resulted in 3160 possible pairs (one profile of VOCs for each of the four biological specimens from twenty individuals). Table 3 provides a summary of the resulting matching errors among the four biological specimen types at the correlation thresholds of 0.9, 0.8 and 0.7.

When comparing each sample across all the other samples, regardless of specimen type, 99.6% of the samples were distinguished at the 0.9 threshold, 97.4% of the samples at 0.8, and 90.6% of the samples at 0.7 threshold. There were no cross-correlations recorded between saliva and any other biological specimen type, which reinforces the distinctiveness of saliva odor. There were cross-correlations between hand odor and hair, hand odor and fingernails and hair and fingernails. The highest occurrence of cross-correlations between specimen types was with hair and fingernails with a total of 61 indistinguishable pairs at the 0.7 correlation coefficient threshold. Additionally, these two specimens produced a high occurrence of indistinguishable pairs ($n = 7$) at the 0.9 threshold, which was not seen as readily during the within specimen evaluations. The likeliness that cross-matching between hair and fingernail samples would occur was revealed during the inter-subject evaluation in the preliminary assessment where the cluster of hair and fingernail samples overlapped.

These results demonstrate that matching errors occur more frequently within a specific specimen type rather than between specimen types. The high percentages of differentiation obtained signify that each specimen provides an elevated level of discrimination power with a volatile profile distinct enough for each individual, yet is not easily cross-correlated to other biological specimens.

Table 3
Number of indistinguishable pairs across all specimen types.

	Hand odor			Hair			Fingernails			Saliva		
	0.9	0.8	0.7	0.9	0.8	0.7	0.9	0.8	0.7	0.9	0.8	0.7
Hand odor	2	39	104									
Hair	0	10	50	0	6	21						
Fingernails	0	2	12	7	13	61	1	4	17			
Saliva	0	0	0	0	0	0	0	0	0	2	7	31

4. Conclusion

Different biological specimens, hand odor, hair, fingernails and saliva, were collected and analyzed using a previously optimized SPME–GC–MS method. From over 239 collected samples, a total of 42 volatile organic compounds were detected that were previously reported to be of human origin. Hand odor samples produced the highest number of compounds previously reported to be of human origin with 24, followed by hair samples with 23, saliva samples with 21 and fingernail samples with 18. The compounds detected in the headspace of the biological specimens included seven functional groups: acids, alcohols, aldehydes, esters, hydrocarbons, ketones, and heterocyclic compounds.

Utilizing the statistical method of Spearman rank correlation, the headspace profile from each biological specimen was correlated against the other samples of that specimen type to evaluate the differentiation power within each biological specimen class. From the results obtained, hand odor samples had two matching errors while hair had zero, fingernails had one and saliva had two occurrences of these errors at the 0.9 correlation threshold. The evaluation of hand odor and saliva samples revealed a notable increase of matching errors as the match/no match criterion was lowered. This suggests that these biological specimens generate volatile profiles which provide sufficient stability and variability for differentiation.

The analysis of biological specimens collected from twenty individuals revealed that matching errors occur most commonly within a specimen type rather than between specimen types; however, the VOCs being released by hair and fingernails can possess sufficient similarity to produce a high correlation coefficient (>0.9) between samples. The high percentages of differentiation obtained when comparing each specimen across all other specimens collected signifies that each specimen provides an elevated level of discrimination with a volatile profile distinct enough for each individual.

This study provided the first account into the use of alternative biological specimens, such as hair and fingernails, as a means for the discrimination of individuals by utilizing the human scent released by these specimens. This work was only one component of human scent that required analysis; however, there are still plenty of other facets of human scent that still require evaluation. Therefore, our next publication will present the persistence of human scent VOCs from biological specimens over time. The collection of samples over time will allow for the identification of the baseline VOCs that are specific to an individual. It is only through a time study that endogenous odors can be identified.

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References

[1] M. Rand, Criminal Victimization 2008. 2009. Bureau of Justice Statistics Bulletin.
[2] A.M. Curran, S.I. Rabin, P.A. Prada, K.G. Furton, Comparison of the volatile organic compounds present in human odor using SPME–GC/MS, J. Chem. Ecol. 31 (2005) 1607–1619.
[3] G.A.A. Schoon, A.M. Curran, K.G. Furton, Odor biometrics, in: S.Z. Li (Ed.), Encyclopedia of biometrics, Springer, Secaucus, NJ, 2009, pp. 1009–1014.
[4] A.M. Curran, P.A. Prada, K.G. Furton, The differentiation of the volatile organic signatures of individuals through SPME–GC/MS of characteristic human scent compounds, J. Forensic Sci. 55 (2010) 50–57.
[5] A.M. Curran, C.F. Ramirez, A.A. Schoon, K.G. Furton, The frequency of occurrence and discriminatory power of compounds found in human scent across a population determined by SPME-GEMS, J. Chromatogr. B-Anal. Technol. Biomed. Life Sci. 846 (2007) 86–97.
[6] K. Saga, Structure and function of human sweat glands studied with histochemistry and cytochemistry, Prog. Histochem. Cytochem. 37 (2002) 323.
[7] M. McKinley, V. O'Loughlin, Cartilage and Bone Connective Tissue. Human Anatomy, McGraw-Hill, New York, 2008, pp. 145–170.
[8] A. Schoon, R. Haak, K9 Suspect Discrimination, Detselig Enterprises Ltd., Calgary, Alberta, Canada, 2002.
[9] A. Faller, M. Schunke, G. Schunke, The skin and its appendages, in: The Human Body: An Introduction to Structure and Function, Thieme, Stuttgart, 2004, pp. 663–672.
[10] R. Smallegange, N. Verhulst, W. Takken, Sweaty skin: an invitation to bite? Trends Parasitol. 27 (2011) 143–148.
[11] N. Goetz, H. Burgaud, C. Berrebi, P. Bore, Analysis of the lipid content of single hair bulbs. Comparison with the content of the sebaceous gland with surface lipids, J. Soc. Cosmet. Chem. 35 (1984) 411–422.
[12] A. Ostrovskaya, P.A. Landa, M. Sokolinsky, A.D. Rosalia, D. Maes, Study and identification of volatile compounds from human skin, J. Cosmet. Sci. 53 (2002) 147–148.
[13] G. Horvath, J. Chilo, T. Lindblad, Different volatile signals emitted by human ovarian carcinoma and healthy tissue, Future Oncol. 6 (2010) 1043–1049.
[14] U.R. Bernier, D.L. Kine, D.R. Barnard, C.E. Schreck, R.A. Yost, Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Indentification of volatile compounds that are candidate attractants for the yellow fever mosquito (*Aedes aegypti*), Anal. Chem. 72 (2000) 747–756.
[15] Z.-M. Zhang, J.-J. Cai, G.-H. Ruan, G.-K. Li, The study of fingerprint characteristics of the emanations from human arm skin using the original sampling system by SPME–GC/MS, J. Chromatogr. B 822 (2005) 244–252.
[16] P. Prada, A. Curran, K. Furton, The evaluation of human hand odor volatiles on various textiles: a comparison between contact and non-contact sampling methods, J. Forensic Sci. 56 (2011) 866–881.
[17] D.T. Hudson, A.M. Curran, K.G. Furton, The stability of collected human scent under various environmental conditions, J. Forensic Sci. 54 (2009) 1270–1277.
[18] A. Curran, R. Stockham, B. Warren, B. Eckenrode, Human scent evidence: scientific support of canine operations and teaching an old dog new tricks, in: Research Partnership Program of 34th Annual Symposium on Crime Laboratory Development, 2006.
[19] M. Harkey, Anatomy and physiology of hair, Forensic Sci. Int. 63 (1993) 9–18.
[20] J.V. Goodpaster, B.C. Drumheller, B.A. Benner, Evaluation of extraction techniques for the forensic analysis of human scalp hair using gas chromatography/mass spectrometry (GC/MS), J. Forensic Sci. 48 (2003) 299–306.
[21] D. de Berker, B. Forslind, The structure and properties of nails and periungual tissues, in: B. Forslind, M. Lindberg (Eds.), Skin, Hair and Nails: Structure and Function, Marcel Dekker, Inc., New York, 2004, pp. 409–464.
[22] S. Chiappin, G. Antonelli, R. Gatti, E.F. De Palo, Saliva specimen: a new laboratory tool for diagnostic and basic investigation, Clin. Chim. Acta 383 (2007) 30–40.
[23] P. De Almeida, A. Gregio, M. Machado, L. Azevedo, Saliva composition and functions: a comprehensive review, J. Contemp. Dent. Pract. 3 (2008) 72–80.
[24] M. Kusano, E. Mendez, K.G. Furton, Development of headspace SPME method for analysis of volatile organic compounds present in human biological specimens, Anal. Bioanal. Chem. 400 (2011) 1817–1826.
[25] J.G. Kostelc, G. Preti, P.R. Zelson, N.H. Stoller, J. Tonzetich, Salivary volatiles as indicators of periodontitis, J. Periodontal Res. 15 (1980) 185–192.
[26] R. Hunt, Benefits of scent evidence, FBI Law Enforcement Bulletin (1999) 18.
[27] J. Miller, J. Miller, Statistics and Chemometrics for Analytical Chemistry, Pearson Edition Limited, Harlow, 2005.
[28] J. Almirall, M. Cole, G. Gettinby, K. Furton, Discrimination of glass sources using elemental composition and refractive index: development of predictive models, Sci. Justice 38 (1998) 93–100.
[29] E. Rodriguez-Celis, I. Gornushkin, U. Heitmann, J. Almirall, B. Smith, J. Winefordner, N. Omenetto, Laser induced breakdown spectroscopy as a tool for discrimination of glass for forensic applications, Anal. Bioanal. Chem. 391 (2008) 1961–1968.
[30] C. Acevedo, E. Sanchez, J. Reyes, M. Young, Volatile profiles of human skin cell cultures in different degrees of senescence, J. Chromatogr. B 878 (2010) 449–455.
[31] M. Gallagher, C.J. Wysocki, J.J. Leyden, A.I. Spielman, X. Sun, G. Preti, Analyses of volatile organic compounds from human skin, Br. J. Dermatol. 159 (2008) 780–791.
[32] N. Goetz, G. Kaba, D. Good, G. Hussler, P. Bore, Detection and identification of volatile compounds evolved from human-hair and scalp using headspace gas chromatography, J. Soc. Cosmet. Chem. 39 (1988) 1–13.
[33] S. Haze, Y. Gozu, S. Nakamura, Y. Kohno, K. Sawano, H. Ohta, K. Yamazaki, 2-Nonenal newly found in human body odor tends to increase with aging, J. Invest. Dermatol. 116 (2001) 520–524.
[34] J. Logan, M. Birkett, S. Clark, S. Powers, N. Seal, L. Wadhams, A. Mordue (Luntz), J. Pickett, Identification of human-derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes, J. Chem. Ecol. 34 (2008) 308–322.
[35] A. Natsch, H. Gfeller, P. Gygax, J. Schmid, G. Acuna, A specific bacterial aminoacylase cleaves odorant precursors secreted in the human axilla, J. Biol. Chem. 278 (2003) 5718–5727.
[36] M. Phillips, Method for the collection and assay of volatile organic compounds in breath, Anal. Biochem. 247 (1997) 272–278.
[37] S. Riazanskaia, G. Blackburn, M. Harker, C. Thomas, The analytical utility of thermally desorbed polydimethylsilicone membranes for *in-vivo* sampling of volatile organic compounds in and on human skin, Analyst 133 (2008) 1027.
[38] A.A. Vass, R.R. Smith, C.V. Thompson, M.N. Burnett, D.A. Wolf, J.A. Synstelien, N. Dulgerian, B.A. Eckenrode, Decompositional odor analysis database, J. Forensic Sci. 49 (2004) 760–769.

[39] X.-N. Zeng, J. Leyden, A. Spielman, G. Preti, Analysis of characteristic human female axillary odors: qualitative comparison to males, J. Chem. Ecol. 22 (1996) 237–257.
[40] J.V. Goodpaster, J.J. Bishop, B.A. Benner, Forensic analysis of hair surface components using off-line supercritical fluid extraction and large volume injection, J. Sep. Sci. 26 (2003) 137–141.
[41] M. Kusano, E. Mendez, K.G. Furton, Comparison of the volatile organic compounds from different biological specimens for profiling potential, J. Forensic Sci. (2012) 1–11.
[42] B.A. Benner, J.V. Goodpaster, J.A. DeGrasse, L.A. Tully, B.C. Levin, Characterization of surface organic components of human hair by on-line supercritical fluid extraction-gas chromatography/mass spectrometry: a feasibility study and comparison with human identification using mitochondrial DNA sequences, J. Forensic Sci. 48 (2003) 554–563.
[43] Y. Masukawa, H. Narita, G. Imokawa, Characterization of the lipid composition at the proximal root regions of human hair, J. Cosmet. Sci. 56 (2005) 1–16.
[44] I. Duvel, H. Chun, D. Deppa, P. Wertz, Analysis of hair lipids and tensile properties as a function of distance from scalp, Int. J. Cosmet. Sci. 27 (2005) 193–197.
[45] A. Lochner, S. Weisner, A. Zlatkis, B.S. Middleditch, Gas-chromatographic mass-spectrometric analysis of volatile constituents in saliva, J. Chromatogr. 378 (1986) 267–282.