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REVIEW

Exploring the feasibility of bioaerosol analysis as a novel fingerprinting technique

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Abstract The purpose of this review is to investigate the feasibility of bioaerosol fingerprinting based on current understanding of cellular debris (with emphasis on human-emitted particulates) in aerosols and arguments regarding sampling, sensitivity, separations, and detection schemes. Target aerosol particles include cellular material and proteins emitted by humans, animals, and plants and can be regarded as information-rich packets that carry biochemical information specific to the living organisms present where the sample is collected. In this work we discuss sampling and analysis techniques that can be integrated with molecular (e.g. protein)-detection procedures to properly assess the aerosolized cellular material of interest. Developing a detailed understanding of bioaerosol molecular profiles in different environments suggests exciting possibilities of bioaerosol analysis with applications ranging from military defense to medical diagnosis and wildlife identification

Keyword Bioaerosols · Skin debris · Fingerprinting · Identification

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Introduction

Bioaerosols are diverse and complex dispersed particles that are either living or of biological origin. These include viruses, pollen, fungal spores, bacteria, and debris from vertebrates, including humans, and other biota (plants, insects, etc). These particles range from ~10 nm to 100 μm [1], and their existence has been recognized for well over a century [2]. Currently, the central topics of bioaerosol studies are health hazards, effects on the atmosphere, terrorism detection, and global climate.

Over the past decade, several studies focused on molecular and isotopic markers that can be used to track bioaerosols, especially for tracing particles released from soils and a variety of agricultural environments [3–5]. Molecular marker studies have mainly focused on organic marker compounds, for example saccharides, alkanes, and steroids for tracing soil dust and plant bioaerosols [4, 5]. Beyond these studies, viewing animal and human bioaerosols as an information-rich marker of its source has not been seriously considered. Reasons for this absence are lack of sufficient (bio) analytical capabilities and poor understanding of the biochemical fingerprints likely to be present in this type of debris. Considering the body of evidence that does exist indicating abundant cellular material and proteins in the atmosphere [6–8], this is somewhat surprising—limited analytical capabilities notwithstanding. Even though there is imperfect knowledge of the "dead" and fragmented biological fraction of particles in the atmosphere, the mere existence of this type of debris creates an opportunity for its use in many potential applications. Living organisms, including humans, constantly release a surprisingly large amount of dead skin cells and fragments into the environment [9]. As analytical capabilities are improved and are focused on the characterization of this fraction (and the living fraction), detailed biochemical information will be found within the

aerosol. This has the potential to significantly affect fields ranging from biochemical forensics and biodiversity studies to medical profiling and environmental studies.

Accurately characterizing bioaerosol to differentiate its source seems to be fighting against the basic concept that many biological structures and metabolic pathways are common to all humans (and many other species) resulting in apparently common biochemical profiles. However, and importantly, structures and pathways exhibit the extensive polymorphisms and divergent post-translational modifications (PTMs) that reflect individual genetics, familial and tribal background, personal history, living environment and health. This variability yields an array of biochemical fingerprints indicative of these polymorphisms and modifications. Analogous to facial-recognition and more recent technology, for example "odorprint," the idea is not to rely on a specific feature (or compound) for recognition, but to obtain multi-component profiles that reflect individuality [10]. Furthermore, it is possible that the concentration, degradation, and type of aerosolized material of interest depends on time since human presence, and potentially this information can be used to time-stamp human and individual occupancy. By exploiting the capabilities of current and emerging analytical technology it is likely that interpretable patterns can be obtained.

The purpose of this review is to discuss the role of bioaerosols, with a focus on bioparticles of human origin, as carriers of biochemical information. We also discuss analysis needs and challenges based on the current state of knowledge for the study of biological aerosols. This work is not intended to provide an exhaustive review of previous studies on aerosol and bioaerosols, but, instead, to provide examples of how analytical chemistry performed in the field and in the laboratory can shed new light on our understanding and analysis of unexploited biochemical fingerprints contained in aerosolized human debris.

Sources of molecular profiles in bioaerosols

The concept of detecting unique molecular profiles from a biological source is predicated on the premise that a unique pattern exists—whether it can be detected or not. Several lines of reasoning and sources of information suggest that, indeed, this profile is generated and released to our surroundings. A variety of biochemical processes are distinctive for each individual and enough material is released into the environment to potentially be detectable. Skin cells are jettisoned in gram quantities daily and remain suspended as an aerosol for hours to days [9]. Presumably, each of these skin cells retains a biochemical fingerprint of the originator. Here, we review current knowledge about the sources of the molecular profiles most likely to be found in skin debris.

Skin cells: sloughing processes and biochemical profile

Aerosolized skin cells, with other forms of animal debris (e.g. dander), are a type of non-viable bioaerosol. They are generated from viable organisms then released into the air spontaneously, as a consequence of environmental conditions, or some other mechanical disturbance. The existence of aerosolized skin in airborne particulate matter has been recognized for more than three decades [11, 12], and has continued to be investigated and better understood in more recent years [8, 13].

Skin flakes comprise a substantial proportion of the recognizable particles of indoor air. This type of debris is also a major constituent of house dust which is constantly re-aerosolized, enabling the skin flakes to re-circulate in the air mass [13]. Bahadori and coworkers reported that mean concentrations of such dust in the breathing zone (44 ± 3 μg m^{-3}) is more than twice that in ambient air [14]. Popular culture notes the large amount of sloughed cells with an urban myth that suggests bed mattresses double in mass over ten years from dead skin cells and dust mites, although more accurately the true attributed weight increase is approximately 20% [15]. The contribution of these particles to sick building syndrome has also been a major concern [16].

Aerosolized skin cells are the result of continuous regeneration of the epidermis. This structure is the external, uppermost, multilayer compartment of the skin in which cornification (or keratinization, culminating in cell death) occurs resulting in spontaneous detachment (desquamation) of corneocytes [9, 17–19]. The cornification process is the highly organized differentiation of keratinocytes changing from a proliferating cell type in the basal layer of the epidermis to an association of flattened, corneocytes in the outermost layer (stratum corneum, SC) [17]. The SC consists of approximately fifteen layers from which cells are continuously discharged into the environment (Fig. 1). The released corneocytes are dead cells, but form the physical layer that protects the skin (Fig. 2).

Through the desquamation process, a single human sheds approximately one gram of aerosolized skin flakes daily, releasing an estimated 10^7 particles per person per day [13, 20]. The average size of these particles is much smaller than the interweave pores of the most clothing fabrics, enabling the skin flakes to move freely through clothing and be released into airstreams [21, 22]. Previous studies have described most particles circulating in the air as small (less than 1–2 μm in diameter) whereas most skin cells freshly emitted by humans are larger, with diameters of 5–15 μm [11, 23]. Each shed skin flake contains a complex mixture of proteins, lipids, small peptides and other biomolecules that is characteristic of its specific source [13].

The product of epidermal desquamation as a source of biochemical information has been largely ignored.

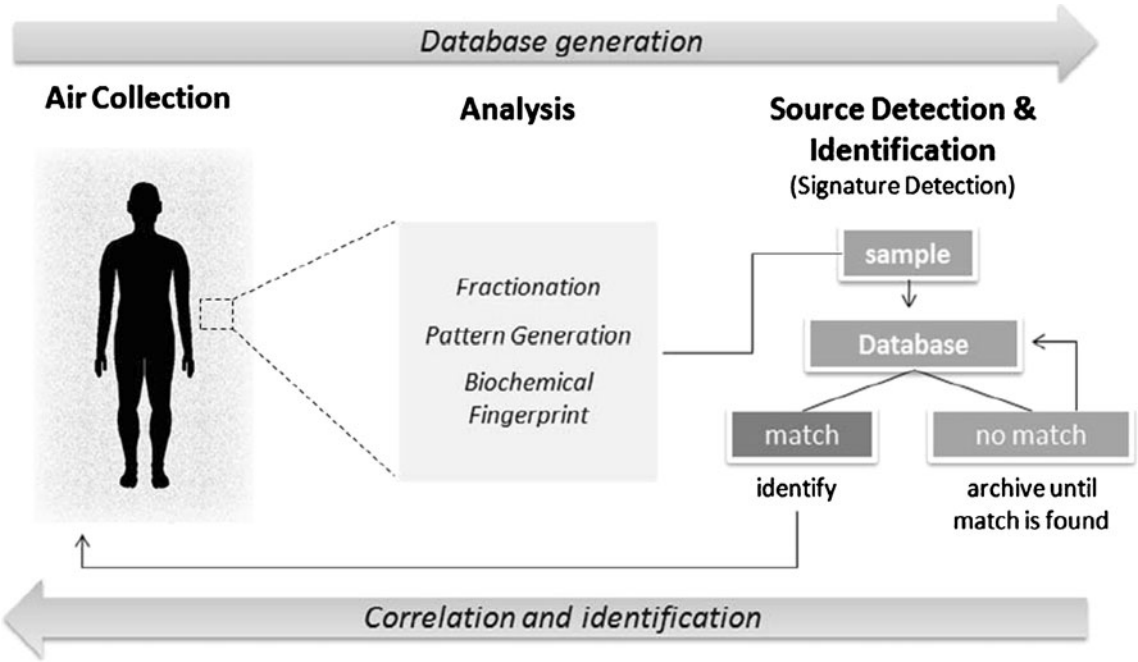

Fig. 1 Overall schematic diagram of bioaerosol technology, consisting in the development of devices for collection and analysis of bioaerosol profile for use as a "fingerprint". Target bioaerosol particles, for example dead skin cells shed by humans, occur pervasively as suspended aerosolized particulates that contain biochemical information unique to their source. This information, most likely in the form of DNA and protein polymorphisms, could be exploited to obtain a biochemical profile from a location of interest. Signal analysis involving biometric comparison of profiles with those in a database can provide insight into potential and/or actual presence of individuals in a particular area. The development of such technology will require integration and refinement of existing advanced technology for aerosol sample collection and automated biochemical analysis

Furthermore, aerosolized skin cells have not been regarded as profile carriers of the individual from which they originate. As a result, there has been little interest in the biomolecules associated with human skin (or with animals)—how these molecules differ with age, conditions, and identity of the source, and their evolution post-desquamation. Some studies have addressed ethnic differences in the desquamation process, although results remain inconclusive and most of the skin properties studied (e.g. water content, pH gradients) are not applicable to analysis of aerosolized skin cells [24].

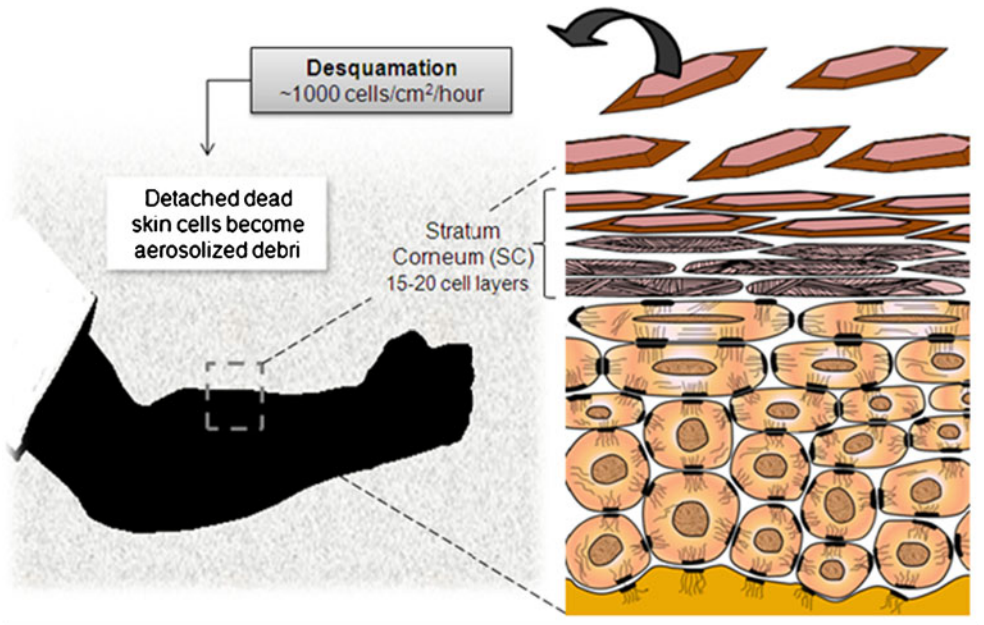

Fig. 2 Diagram of the epidermal desquamation process [9]. Humans shed approximately billions of cells per day. Each skin flake is the product of a program of differentiation that ends as easily-detachable corneocytes. As desquamation occurs, dead skin cells settle slowly in the air providing an unexploited opportunity to obtain biochemical information unique to their source from aerosolized samples

Nevertheless, examples of biomolecular profiling from shed material (e.g. bulk amino acid of detached feathers for bird speciation [25]) demonstrate the information that can be gleaned from detached bioparticles.

A trivial argument can be made that unique biomolecular systems result in unique biophysical structures and these structures can be used for fingerprinting purposes. This is the assumption underlying many microscopic and histological strategies. In terms of an analytical approach used to extract the necessary molecular information, the structure of corneocytes alone may initially be used to categorize the source. Skin cells obtained by stripping methods (removing layers of cells on adhesive coated tape) and other traditional techniques (e.g. detergent scrubs) show that the geometry of corneocytes can be correlated with the type of skin epidermis [26, 27], its age [28, 29], and its health [30]. These correlations are for dermatological treatment [28, 29, 31] and their applicability to the proposed system of bioaerosol fingerprinting is not known. Other studies show that women shed larger skin cells than men, and that size increases with age [26]. Some studies have addressed environmental effects (e.g. solar exposure) [32] on corneocytes. However, many inconsistencies exist among the reports [9]. Further, there are no studies of corneocyte geometry changes from cells collected from air samples. The extent to which corneocyte geometry assessment will be valuable to bioaerosol fingerprinting is unknown, but it does provide a valuable line of inquiry.

DNA

Since the mid-1980s, advanced technology has enabled the DNA-typing of biological material to become the most powerful tool for identification purposes. The DNA profile from an individual is largely unique and identification can be made from one profile only. For DNA typing, short tandem repeats (STRs) polymorphism provides the basis of personal identification [33–35].

Traditionally, DNA-typing has been a forensic tool routinely used for analysis of biological fluids, tissues, and uprooted hair. More recent studies have reported DNA typing from fingerprints and skin debris left by even a single skin contact on objects and clothes [35–37]. Van Oorschot and Jones reported that substantial transfer of material (approximately 1–75 ng DNA) occurs during initial contact [37]. Kisilevly and Wickenheiser profiled DNA of skin cells transferred through handling [38]. They reported that the amount of DNA transferred to a substrate depends on the handler, with some individuals being “good” epithelial cell donors (sloughers) whereas others individuals are “poor” epithelial cell donors (non-sloughers). Obviously, the probabilities of obtaining a full DNA profile are maximized with the former kind [38, 39]. Schulz and Reichert reported preliminary tests showing successful DNA typing in archived fingerprints that have been manipulated using soot powder, magnetic powder, and scotch tape [36, 40]. Reports such as this demonstrate the possibility of DNA profiling for samples that have been stored and relatively contaminated. DNA traces, such as those left in fingerprints, can also be easily wiped or brushed off surfaces [35], suggesting they can also be easily aerosolized.

Further, the DNA typing of a single human dandruff particle was demonstrated by Herber and Herold [41]. Dandruff can be a constituent of bioaerosols and is derived from the horny layer of the skin where the cells do not completely differentiate; its aggregates contain nuclei. This is in distinct contrast to fully cornified cells (emitted skin flakes) from which the nuclei have completely disappeared and no nuclear fragments or remnants remain [42]. In their study, Herber and Herold reported an estimated range of 0.8–1.5 ng DNA per dandruff flake. Additionally, they were able to achieve successful STR analysis for 90% of their samples. In loosely related results, DNA profiling for identification of viruses and bacteria from bioaerosol samples has been reported in the literature. These results support the general idea that emitted skin (and other human-related biota) particles can serve as a source of a biochemical profile if DNA analysis is used.

Limiting this line of reasoning are fingerprint DNA-typing studies showing that more than 1 ng of DNA (equivalent to 200 cells) is required (DNA typing in single cells has been demonstrated, but only for the buccal cell type [43]). Picogram levels of DNA have also been reported to provide satisfactory DNA typing. However, analysis of minute samples is highly complicated by contamination issues [44], which is a significant concern for aerosolized samples. Degradation and environmental effects will further limit the usefulness of DNA as an information source. Currently, there are no reports of DNA typing being applied to aerosolized human skin cells for identification purposes. Further, considering that fully cornified cells do not contain any DNA [42]; the probability of finding useful amounts of DNA is not favorable.

Protein variants and polymorphisms

Cellular proteins (and other biomolecules) contained within aerosolized skin debris can, presumably, be used for identification purposes but, unlike other identifiers, for example DNA and fingerprints, can also give information about the individual’s state of health, where she or he has been living, and under which environmental conditions. Because protein sequences are linked to gene sequences, proteins can be regarded as a more characteristic biomarker of an individual than other type of molecule (e.g. lipids). Polymorphisms in

DNA coding regions are precisely reflected in the polymorphisms of proteins and their derivatives. The same information that enables DNA analysis to reflect genealogy, family, and individuality may thus be obtained from specific proteins.

In humans, the SC contains 75–80% proteins (dry weight) [45]. Protein analysis in the SC has been performed mainly to address desquamation abnormalities. Furthermore, variability in the expression of SC proteins may or may not exist among different individuals, but in either case, the extensive literature documenting protein and DNA polymorphisms [46, 47] suggests that differences in expression are likely and, therefore, proteins make good candidates for obtaining molecular profiles. Of specific interest are reports of inter-individual variation of the epidermal keratin proteins [48] that occur in dead skin cells.

Protein profiling has already been demonstrated with automated systems capable of detecting aerosolized bacterial cells and spores [49, 50]. In a moderately warm and humid environment the largest human aerosol particles settle first and are digested first by the fungus *Aspergillus repens* and then by dust mites [51]. Thus, the concentrations and types of aerosolized proteins depend on time since human presence, and potentially this information can be used to time-stamp human and individual occupancy.

Protein variants among populations are already of much interest in emerging fields such as personalized medicine [52]. Beyond protein quantification, the search for disease biomarkers has involved intensive study of protein polymorphism and posttranslational modifications such as oxidation, glycosylation, and truncation within the products of a single gene between healthy and unhealthy individuals. Borges and coworkers state that these types of modification "extend the diversity of human gene products dramatically beyond 20,000–25,000 genes in the human genome" [53]. Quantifying these variants by means of the methods of proteomics and mass spectroscopy (MS) has revealed the immense diversity of proteins (and protein modifications) in samples such as human plasma. With other studies, these assessments have led to the field of population proteomics [53–56]. Pioneers in this field have already proposed the creation of protein-diversity databases in which protein variants are indexed relative to age, sex, race, geographical region, disease, and other useful metrics [57]. As efforts toward expanding the knowledge of protein variability in humans (and other organisms) continue, parallel progress is expected that other fields, for example fingerprinting techniques based on biomolecular profiles. Unlike other areas, protein fingerprinting would not require the complete isolation and characterization of low-abundance proteins or variants. Instead, the sole acquisition of protein profiles generated by these variabilities can become the basis of obtaining a biochemical pattern for database generation and identification purposes.

Keratin polymorphisms

Skin cells consist of more than 80% keratins cross linked to other cornified proteins [42]. Aside from being abundant in skin debris, these are the most appropriate protein target when seeking individual variability. Keratins consist of more than 20 polypeptides (K1–K20) that are classified into relatively acidic Type I (K9– K20) and neutral-to-basic Type II (K1–K8) [58]. All epithelial cells typically express at least one Type I and one Type II keratin. For example, K4 and K13 are characteristic of the buccal mucosa and K1 and K10 are found on the dry surface of the skin [59].

Keratin is the major non-aqueous component (*w*/*w*) of the SC. Dead skin cells mostly consist of keratin intermediate filaments (KIFs) which are keratin structures that form the cytoskeleton of all cells [17]. As skin flakes are spontaneously released into air streams as a result of the desquamation process, human keratins become part of the bioaerosol in areas where humans are (were) present. The epithelial human keratin K10, derived from shed human skin and its associated bacteria, has recently been determined by Fox and coworkers as the most abundant protein in airborne dust of both occupied and unoccupied school rooms [60]. In addition, keratins have also been identified as a common contaminant in protein analysis such as gel electrophoresis and MS. It is believed that the source of these keratins is the laboratory air in which skin particles can be pervasive [60].

Heterogeneity in keratin structures has been reported for both animal [61] and human [48, 62] subjects. Polymorphisms in keratin genes give rise to protein heterogeneity in different types of epithelium, including epidermis. More than a decade ago, Mischke and Wild identified these polymorphisms as important factors that could affect forensics sciences [62]. Although their study mainly emphasized keratin polymorphism from epidermis samples obtained by means of surgery, they also reported inter-individual variation in the processed keratins from the layers of the SC.

Mischke and Wild showed that polymorphic keratins were present in human epidermis and some were identified among the individuals for specific constituent protein subunits, but this pattern does not correlate with sex, age, or ethnic origin [62]. In their study, they concluded that keratin polymorphisms can generally be expressed in all human epithelial cells capable of expressing keratins 1, 4, 5, and 10 [62]. As previously mentioned, keratins 1 and 10 are expressed in the dry surface of the skin. Therefore, keratin polymorphisms are expected to exist in the dead skin cells that eventually become detached from the skin surface to become aerosolized human particulates in the environment.

In 1992, Korge and coworkers reported that human K10 is more polymorphic than was previously thought. Their results confirmed that the human K10 intermediate filament protein is polymorphic in amino acid sequence and in size.

This was determined by using PCR amplification followed by sequence analysis on DNA. They observed variations in the V2 subdomain near the C-terminus in glycine-rich sequences with variations of as much as 114 base pairs (38 amino acids), with all individuals having one or two variants. The K10 polymorphism is restricted to insertions and deletions of the glycine-rich quasipeptide repeats that form the glycine-loop motif in the terminal domain. They also reported that the polymorphisms can be described by simple allelic variations that segregate by normal Mendelian mechanisms [48].

To some extent keratin proteins have been neglected because cytosolic proteins are less difficult to analyze [63]. However, and clearly, there are potential benefits from understanding keratins and their polymorphisms (and variants), not only for diagnostic tools and other already recognized applications, but pattern generation. Some of the potential benefits of keratin analysis for the applications proposed here include their abundance in aerosolized material, the evidence of polymorphism, and their structural robustness in comparison with other protein material. The robustness of keratins may, in fact, be the most important factor in obtaining crucial information from aerosolized particles in an environment in which other types of biochemical "stamps" would be highly degraded. It is important to note that the studies mentioned above did not particularly use aerosolized skin cells as their samples, but most certainly traces of these profiles remain after the desquamation process. The development of a bioaerosol fingerprinting technology relies heavily on understanding how these keratin polymorphisms remain in skin cells post-desquamation and finding strategies for practical detection from aerosolized human debris.

Other classes of molecular targets

Aside from DNA and proteins, individualized information from aerosolized human debris may also be obtained from SC lipids or more exotic sources, for example skin bacteria.

Lipids components were first used for general identification of skin flakes approximately three decades ago. Analysis of surface fat material in recovered airborne particulate matter enabled identification of skin particles in material that was previously dismissed as "dust of unknown origin" [12]. Regional variations in SC lipids have been addressed by many studies [64]. However, the lipid makeup of dead skin cells post-desquamation is not well-known. Consequently, inter-individual variability in lipid content from skin flakes has not been addressed, and understanding of how this composition varies may be used to generate additional molecular fingerprints for aerosolize material. Presumably, lipids in aerosolized skin cells may not be as readily available in comparison with other biomolecules, because the mixture of ceramides, cholesterol, and fatty acids is primarily located in the intercellular spaces of the SC [65] rather than within the individual corneocytes.

The human skin also harbors complex microbial ecosystems that seem to be unique to each individual, making them a potential source of a biochemical fingerprint. However, little is known in detail about its species composition. Recently, Gao et al. reported the variability of bacteria species existing in the surface of the skin, after use of PCR-based methods [66]. The biota of superficial human skin is highly diverse among individuals, with a few conserved and well represented genera, but otherwise low interpersonal consensus. The importance of this study in the context of this work is that when individual variability in skin biota composition is better understood it can potentially be another source for pattern recognition. This idea of using bacteria as a source of information is also supported by the results obtained by Tham and Zuraimi, who concluded that the main contributors of viable bacteria in indoor environments are in fact humans [23].

It is important to keep in mind that environmental conditions such as temperature, humidity, and exposure to light, and host factors including genotype, health, gender, immune status, and cosmetic use may affect microbial composition, population size, and community structure [66]. These factors may increase the complexity of the biota and its variability, but also suggests a better, more information-rich source of patterns—if interpretable.

Molecular profile success stories

Specific cases involving the use of molecular profiles for identification or differentiation offer some insight into molecular fingerprinting strategies. For example, profiles of mammalian and reptilian keratins (horn, hoof, and tortoiseshell) have been used to differentiate a broad range of sea turtles and bovid species. These studies involved the use of Fourier-transform Raman spectroscopy, diffuse reflectance Fourier-transform spectroscopy (DRIFT), and discriminant analysis to distinguish and identify the species-specific keratins [67]. Spectral library searches enabled comparison of the unknown with a set of possible matches (species population) [68]. Furthermore, the level of statistical confidence for each specific assignment could be calculated.

DNA-typing has enabled the detection and identification of viruses and bacteria in bioaerosol samples. Peccia and Hernandez have reviewed emerging technology incorporating PCR-based approaches with aerosol science for the identification, characterization, and quantification of microorganisms for both indoor and outdoor environments [69]. Aside from microorganism detection in large-scale aerosol collections [70, 71], the identification of virus and bacteria

from aerosolized DNA has also been demonstrated in smaller sampling devices. Pyankov and coworkers reported a personal sampler that could rapidly detect viable airborne microorganisms [72]. Identification of the influenza and vaccinia viruses [73] and the mumps and measles viruses [74] from bioaerosol samples has also been reported. Targeted PCR analysis is not the most appropriate approach for obtaining molecular fingerprints from skin flakes shed by humans. However, these analyses overcame substantial contamination by a large number of microorganisms and much other material commonly found in ambient air. This demonstrates that large and complex backgrounds such as these do not preclude molecular pattern recognition strategies from being successful [72–74].

Field fractionation, capillary electrophoresis, and microfluidic electrophoresis techniques have been used to resolve a variety of microorganisms, and smaller particles, for example viruses and DNA fragments [75–78]. An automatic microfluidic device for detection of aerosolized bacterial and spores on the basis of protein profiles has also been demonstrated [50]. Similar technology for protein profiling of viruses, although not in aerosol samples, has been also reported by Fruetel and coworkers [49]. This technology and other procedures could be used to obtain protein profiles of the aerosolized human debris of interest [79].

Another powerful example of molecular fingerprinting from complex samples is the detection of airborne profiles of humans on the basis of volatile organic compounds (VOCs) of human scent. By use of this technology the distinctive odor characteristics that enable canine scent discrimination are exploited to differentiate primary odors among human subjects. The "primary odor" contains the constituents that are persistent over time, irrespective of diet or environmental factors (including exogenous sources, for example lotions and soaps) [80]. Using the volatile organics that make up the human scent as means of medical diagnosis and as markers of genetic individuality has been demonstrated within the past decade [80–82]. A variety of extraction techniques (e.g. solid-phase microextraction) have been combined with GC–MS and pattern-recognition strategies in a search for quantitative differences in qualitatively similar profiles. Extensive surveys of odor-producing VOCs including acids, alcohols, aldehydes, hydrocarbons, esters, ketones and nitrogen-containing compounds furnished reproducible and individualized profiles based on the relative ratio of these components [82]. Similarly to other identification systems, they stated how these profiles could be stored in a searchable database for use as a biometric measure. By performing comparisons via Spearman rank correlations and narrowing the compounds considered for their profiles they were able to achieve greater individualization and discrimination [82]. Although the existence of valid VOCs profiles has been demonstrated, this strategy has significant limitations. Airborne profiles rely upon volatile compounds which are relatively small in number, can be confused with background compounds, disperse rapidly, and have poor detection limits.

Bioaerosol analysis and pattern-recognition strategies: needs and challenges

When considering which of the various biomaterials described in previous sections to use for detection and identification, it is important to understand what information can be collected from each type (Table 1). For example, the detection of bio-terrorism threats focuses on the collection and analysis of bacterial and viral particles specifically those that maintain their pathogenicity. Targeted biomolecules should be chosen for ease of detection in order to maximize unique identification and detectability, with identification either by detection of a single component or by comparing a collection of components.

The first hurdle to utilizing the information borne in aerosolized biomolecules and cells is to capture sufficient amounts of material to generate statistically distinguishable and reproducible patterns for classification. Steps toward this objective have been taken in research described in previous sections [83–86].

However, a plethora of complicating organism-specific and environmental variables will require refinement of sampling and detection techniques, for example, concentration of target proteins in the air range largely depending on the environment measured. Current measurements of total aerosolized protein range in concentration from 0.1 to 3 $\mu g\ m^{-3}$ (up to 8 $\mu g\ m^{-3}$ for physically induced samples) in the presence of humans, and can vary from 0 to 2 $\mu g\ m^{-3}$ for outdoor situations with limited human presence [8, 69, 87]. Within this amount of total protein are thousands of fractions of different individual species and mixtures with different magnitudes of individual concentration. The detection limits for individual molecular species of proteins is, conservatively, approximately 100 pg, depending on the protein purification and detection strategy used. Detection schemes focusing on the DNA in specific sample types (e.g. buccal) use as few as a single cell to functionally create a DNA profile [43]. These limits of detection illuminate some of the challenging aspects of accurately detecting biomolecular sub-fractions by use of current technology. Expanding and improving current methods will lead to a wider range of probe biomolecules being detectable, and the necessary concentrations for detection will be reduced.

Analytical strategies for bioaerosols have been extensively reviewed in the literature [1, 83, 88–92]. Traditionally, bioaerosols are analyzed by culture, microscopy, biochemistry, immunochemistry, and flow cytometry. Other techniques have also been used to a lesser extent, for example a fluorescent

Table 1 Possible sources of biochemical profiles in bioaerosols from various types of sample

Sample type	Skin debris	Fur/hair	Feathers	Scales	Insect debris	Plant debris
DNA	Occasionally, truncated	Only in root-bulb	Yes	Occasionally, truncated	Yes	Yes
RNA	Yes	Not beyond root-bulb	Unlikely	Unlikely	Yes	Yes
Proteins	Yes, structural (e.g. keratin)	Yes, structural (e.g. keratin)	Yes, structural (e.g. keratin)	Yes, structural (e.g. keratin)	Structural (e.g. chitin)	Yes, structural
Nucleic acids	Small amounts	Not beyond root-bulb	Small amounts	Small amounts	Yes	Small amounts
Lipids	Yes, large amounts	No	No	No	Yes	Yes
Glycosylated species	Yes	No	No	No	Likely	Likely
Amino acids	Yes	Yes	Yes	Yes	Yes	Yes

aerodynamic particle sizer (FLAPS), fluorescence in-situ hybridization (FISH) [78], and laser-induced fluorescence (LIF) [93]. Spectroscopic techniques, for example Raman spectroscopy [94, 95] and Fourier transformer infrared spectroscopy [96], have also been used for characterization of bioaerosols. Emerging capillary electrophoresis approaches are also promising for generating biomolecular profiles from aerosolized human debris [97, 98].

The use of MS for analysis of aerosolized material of biological origin has already been reported [99–103], including the bioaerosol mass spectrometer (BMAS) developed for the detection of microorganisms [104, 105]. Ariya and coworkers recently reviewed the compounds used to identify the specific bioaerosol sources that were separated and detected by GC–MS strategies [1]. They report that detection limits for individual compounds are typically in the low pg m^{-3} range for GC and LC–MS and that even overlapping compounds in very complex mixtures could be successfully interpreted [1]. The profiles used to detect microorganisms correspond to the mass spectrum—predominantly that of peptide and proteins—acquired for a particular organism under a variety of conditions, which can then be matched to the masses predicted for organisms with sequenced genomes [106, 107].

To generate a pattern related to the molecular constituents of bioaerosols, spectrometric, spectroscopic, and/or separation (including molecular recognition) strategies can be used. The underlying principle is to be very inclusive toward any technique or approach that can help differentiate one sample from another. This can take the form of separated chromatographic or electrophoretic peaks, molecular recognition strategies, different absorption or emissions at different wavelengths, or mass spectrometric analysis. The discerning of complex patterns for identification is well developed within the data-mining community. However, as with any pattern recognition, the accuracy of the analytical technique can affect interpretation—essentially defining the number of values that can be assigned. An approximate estimate of the necessary protein concentrations can be generated by use of a standard assessment of signal to noise. Approximately setting three times the detection limit as the accuracy and the dynamic range at four orders of magnitude suggests that possibly 10^7 unique profiles are available from current strategies. This can also be described as having n number of vectors (fractions) having m discernable values giving $n \times m$ unique solutions for truly random projections. However, real world samples will not give this many profiles, because it is not a random system—this is simply an estimate of the order of magnitude that can be obtained. Pattern-recognition strategies can be viewed as an integral means of combing environmentally and biologically complex samples and separating them into statistically relevant profiles of the source of the material.

For example, chemometric techniques applied to bacterial taxonomy[108] and geographical sourcing of medicinal plants [109] was aided by classification of data into groups via related strategies. Chemometric characterization of aerosol bacteria by LIF has also utilized a variety of approaches, including principal-component analysis, linear discriminant analysis, and hierarchical cluster analysis, to classify the microorganisms according to family, morphology, and Gram-test [110]. These reports exemplify the power of coupling analytical techniques with effective mathematical post-processing to achieve differentiation of molecular profiles among aerosolized samples.

The ability to combine sample collection, analytical treatment, and pattern recognition will define how much information can be garnered, although the specificity of the identification desired dictates the analytical refinement required. For instance, is it sufficient in some applications to discern the simple presence of *any* person? Conversely, it may be desirable to find a specific profile in the presence of other family and clan members, among other complicating profiles.

A more in-depth understanding of bioaerosol material is still required and the performance of specific analytical

techniques remains to be established to enable separation and detection of molecular patterns.

Focusing on human detection, a variety of samplers, personal and static, could find use in specific settings. For example, for defense applications a static sampler could be used in remote areas to monitor the presence and movement of adversary troops. Personal samplers may be more appropriate if a new setting must be screened. Application could include a rescue mission for trapped miners in which a more portable device will be more beneficial in the search for aerosolized human fingerprints in potential sites were individuals could be trapped. Both types of sampler will require subsequent separation of the debris of interest from other background particulates. Potential methods include the application of dielectrophoretic separation to quickly assess the bioparticle portions of interest in a given sample before to further fractionation [98, 111, 112].

Envisaged application of technology

The future capabilities of this technology to perform gross identification would build upon the current possibilities of determining the presence of humans by expanding to distinguishing different types of organism. In addition to determining their presence, looking at the extent of material degradation might also be exploited to determine how much time has passed since the occupation.

We envisage a few different types of device utilizing different levels of technology and with various capabilities—from portable, relatively information-poor devices, to fixed information-rich systems. Some exciting applications of this technology include active and passive TTL (tag, track, and locate), check points (military) and airport security, forensics, healthcare, personnel identification, non-invasive biological and environmental monitoring, archeology and paleontology, anti-poaching, and rescue missions.

Summary and outlook

It may be premature to conclude that aerosolized human and animal debris and the excess protein content of the air can be used to identify individual sources of emitted particles. However, combination of the current findings reported here with the unexploited information that exists in aerosolized human and animal cells is certainly encouraging with regard to their use as “calling cards” left by the biological sources (humans or animals present in the area of air sampling).

The concepts described throughout this review provide new direction and opportunities for research. Research should be directed at identification of the biomolecules within the aerosolized skin cells and other aerosolized debris that provide maximized differentiation among sources. Strategies to exploit the information from these molecules must be examined and improved, in order to take full advantage of the biochemical profiles contained within the aerosolized flakes. Understanding of transformations and aging processes of bioaerosols must be further improved to exploit the opportunities for the time-stamp aspects of bioaerosol fingerprinting technology.

There is a critical need for synergism between the various available techniques for sampling, separation, and analysis to achieve the necessary depth in obtaining information from human-emitted bioaerosol particles. This combined effort will enable us to be in a much better position to develop novel state-of-the-art techniques for complete analysis of aerosolized cells.

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