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Opinion

# Skin Metabolomics

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**Skin retains numerous low-molecular-weight compounds (metabolites). Some of these compounds fulfill specific physiological roles, while others are by-products of metabolism. The skin surface can be sampled to detect and quantify skin metabolites related to diseases. Miniature probes have been developed to detect selected high-abundance metabolites secreted with sweat. To characterize a broad spectrum of skin metabolites, specimens are collected with one of several available methods, and the processed specimens are analyzed by chromatography, mass spectrometry (MS), or other techniques. Diseases for which skin-related biomarkers have been found include cystic fibrosis (CF), psoriasis, Parkinson's disease (PD), and lung cancer. To increase the clinical significance of skin metabolomics, it is desirable to verify correlations between metabolite levels in skin and other biological tissues/matrices.**

## Origin of Skin Metabolites

Metabolomics is one emerging field of 'omic' sciences in systems biology, which aims to reveal the metabolites within a biological system [1,2]. Arguably, some notable activities associated with this approach occurred as early as the 1970s [3]. Blood and urine are two biofluids that are frequently sampled and analyzed to obtain clinically relevant information, but there is a growing interest in the metabolomics of unconventional biological matrices, such as skin excretions [4]. Low-molecular-weight compounds present on or within skin originate from sweat, sebum, and protein degradation occurring in the outer layers of skin [5] as well as **interstitial fluid** (see Glossary), which constitutes ~45% of the volume fraction of human skin [6].

Metabolites in sweat are from secretions of **eccrine** and **apocrine glands** (Figure 1A,B). Eccrine gland secretions are released across the luminal cell membranes of the secretory cells as an aqueous fluid without cell disintegration [7]. The secretion contains electrolytes, elements, inorganic ions, amino acids, and other unknown species [8]. Apocrine gland secretions occur via pinching off of secretory cells in the apocrine gland coil [8,9]. This kind of secretion is a viscous fluid containing lipids, proteins, steroids, and ions [8].

Sebum, which is a complex mixture of lipids, is produced and secreted by sebaceous glands (Figure 1C) [10]. Sebum discharge is a holocrine secretion because it is formed by complete disintegration of glandular cells into the follicular duct of pilosebaceous units [10]. Volatile organic compounds (VOCs) on human skin are produced from the secretions of eccrine, apocrine, and sebaceous glands, and the interaction of skin microbiota with these secretions (Figure 1H) [11]. Although the composition of interstitial fluid (Figure 1D) is not yet fully characterized, some researchers have reported that it is similar to that of plasma/serum because of the presence of proteins and lipids. However, the concentrations of interstitial fluid components (e.g., proteins, calcium, magnesium, sodium, and potassium) are generally lower than the concentrations in blood due to their transcapillary flow from blood to the interstitial fluid [6].

Other compounds present on skin (e.g., amino acids, compounds produced by environmental stressors, or metabolites from topically applied drugs or consumer products) are found in its

## Highlights

Skin metabolites are low-molecular-weight compounds found in different components of skin.

The field of skin metabolomics relies on the availability of methods for efficient skin sampling and analysis of metabolites in low-volume specimens (e.g., sweat).

A variety of skin sampling techniques are available as customized approaches, targeting specific applications and addressing impediments of invasive skin sampling.

Skin metabolomics is an emerging translational research tool for biomarker discovery and precision medicine.

Biosensor innovations focus on the detection of alternative biofluids from the skin, aiming to develop non-invasive point-of-care testing devices.

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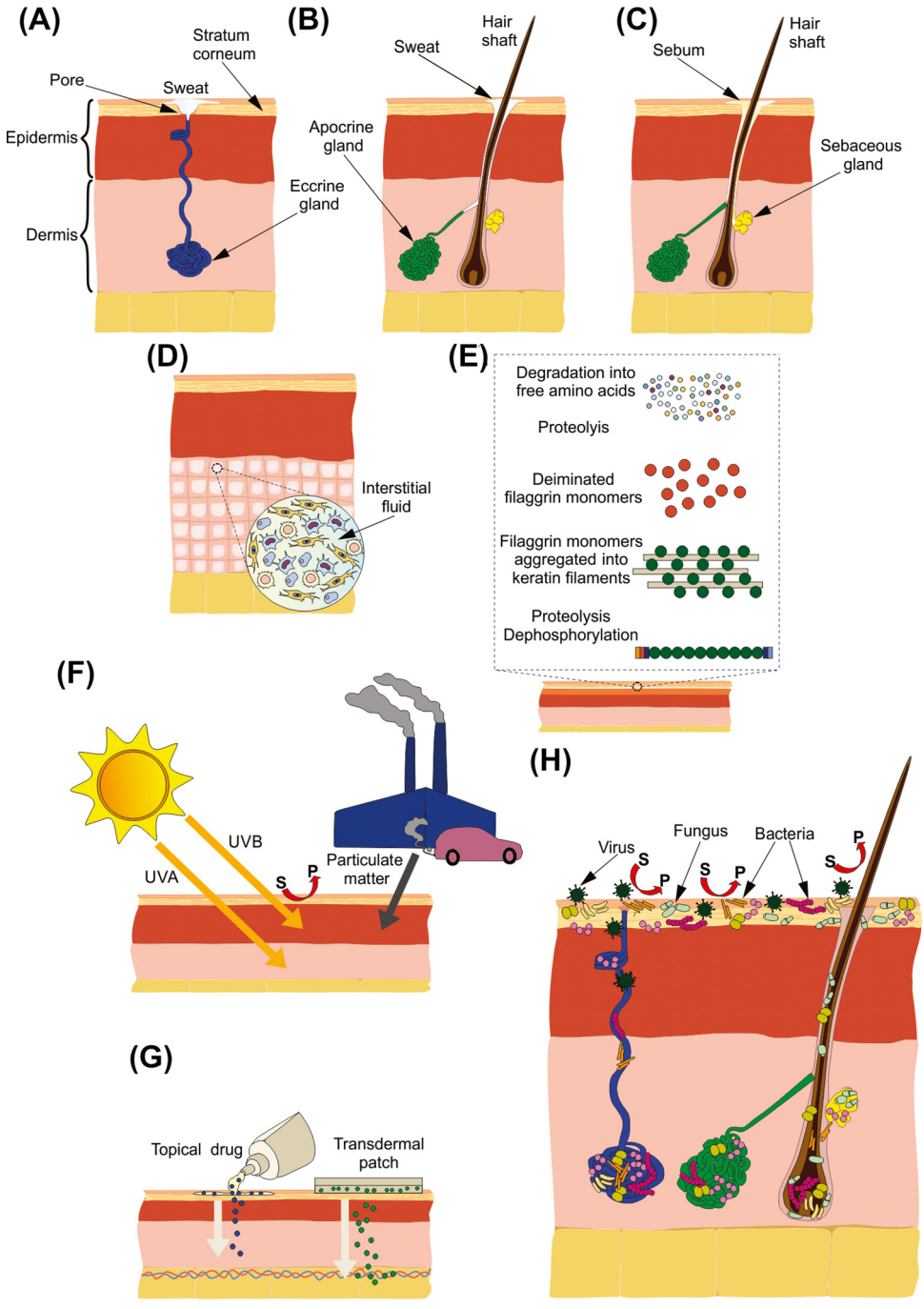


Figure 1. Pathways via Which Metabolites Are Released by Skin, and Exogenous Sources of Skin-Retained Species. Metabolites found on skin originate from various sources. Skin metabolites are detected from the compounds excreted by the skin glands, predominantly eccrine glands (A). Trace amounts of sweat excreted by apocrine glands (B), and sebum secreted by sebaceous glands (C) are also detected in skin excretions. Metabolites that are not excreted onto the skin can be found in interstitial fluid (D) and epidermis (E), particularly the degradation products of profilaggrin in the stratum corneum. Metabolites of skin-retained species from exogenous sources are also detected on the skin. Skin

*(Figure legend continued at the bottom of the next page.)*

## Glossary

**Apocrine gland:** glands that open into the hair canal and develop in areas abundant in hair follicles (e.g., axilla, mammary, perineal, and genital region); respond to emotional stimuli; their secretions are initially odorless but become odorous upon reaction with skin surface bacteria.

**Biopsy:** medical procedure involving removal of tissue from almost any part of the body for further laboratory examination for a disease. Sample preparation is done on the removed tissue sample, such as chemical treatment, freezing, and staining, before examination under a microscope.

**Eccrine gland:** most abundant glands on the surface of the human body, with the highest density in hands and feet; open directly on the skin surface, producing odorless sweat.

**Filaggrin:** class of proteins that specifically interacts with keratin filaments, but not with other types of cytoskeletal protein.

**Hydrogel micropatch:** millimeter-sized disk-shaped sampling medium comprising hydrogels (typically agarose); is embedded in a polymer base, and used to collect skin excretions from the skin surface.

**Interstitial fluid:** fluid found in the spaces around the cells of organisms; comprises water and solutes leaked out by cells of the blood capillaries.

**Iontophoresis:** technique in which a chemical or a medicine is introduced through the skin by the application of a mild gradient of electric current.

**Macroduct:** disposable plastic device for collecting sweat induced by iontophoresis. Sweat flows from the skin into the spiral microbore tubing of the device and is then collected at the open end of the tubing via a dispenser or syringe.

**Mass spectrometry:** analytical technique in which the analyzed compounds in the sample are first transferred to the gas phase and ionized, and then separated in electric and/or magnetic fields according to the mass-to-charge ratios of the gas-phase ions.

**Metabolic fingerprinting:** metabolomics strategy that involves identification and/or recognition of chemical patterns or metabolite fingerprints of samples from different biological status. This approach does not aim for complete metabolite

outer layers (Figure 1E–G). For instance, monomeric **filaggrin** is produced from dephosphorylation and degradation of the insoluble polyprotein profilaggrin in the stratum corneum. Subsequently, filaggrin further undergoes proteolysis to break down and release the component amino acids and their derivatives [12]. Profilaggrin is the major source of free amino acids and their derivatives that principally comprise the natural moisturizing factor (NMF) of the stratum corneum. In fact, the main components (~ 40%) of NMF are free amino acids, including serine, glycine, alanine, histidine, ornithine, citrulline, and arginine [13]. Mutations of the gene encoding filaggrin, which cause skin barrier dysfunction, are genetic risk factors for skin diseases such as ichthyosis vulgaris and atopic dermatitis [12].

## Methodologies for Sampling and Analysis of Skin Metabolites

There exist diverse methodologies for skin specimen collection and identification across different skin metabolome studies (Figure 2). Interest in the development of such methodologies results from the fact that the skin is the largest, most exposed, and, therefore, most accessible organ of the human body. In addition, skin sampling can be customized and, sampling methods have been developed to address specific applications. Skin metabolic profiling can be performed on conventional matrices, such as the layers of the skin (epidermis, dermis, or superficial fat), which need to be obtained invasively [14]. Sampling techniques, such as **biopsy** (Figure 2Ai,ii) and **suction blistering** (Figure 2Av), are conventional techniques in the clinical setting [14]. Skin biopsy is an invasive technique. Depending on the specific skin biopsy mode, specimens can be obtained from or beneath the epidermis (including dermis and superficial fat) [14]. Suction blistering techniques, which are considered less invasive relative to skin biopsy, can be used to collect the epidermis and interstitial fluid [15]. Other techniques, such as microdialysis (Figure 2Aiii) and open-flow microperfusion (Figure 2Aiv), can also be used for sampling interstitial fluid in the skin by implanting thin tubular membranes (for microdialysis) and membrane-free probes with macroscopic openings (for open-flow microperfusion) [16]. Skin biopsy, suction blistering, microdialysis, and open-flow microperfusion are all considered to be patient unfriendly because they are time-consuming, inflict pain, and incur a potential risk of complications [16,17].

For the past few years, minimally invasive to non-invasive *in vivo* and *in situ* sampling techniques have been devised for skin metabolite analysis (Figure 2Av–xii). Different skin-sampling techniques have been developed with features that address some impediments of invasive skin sampling. Unlike conventional techniques, which sample skin tissue, specimens can be collected non-invasively from skin excretions (sweat, sebum, and volatile compounds emanating from the skin) and stratum corneum. The standard sweat sampling methodology involves sweating induction with **pilocarpine** and **iontophoresis** followed by **macroduct** sampling (Figure 2Avi) [9]. In some cases, sweating is stimulated by physical exercise and environmental temperature control [18]. These perspiration-inducing methods can affect the composition of the sampled sweat [19]. Sweat and sebum (including volatile compounds emanating from the skin) are collected by using solvents (or buffer) to wash the skin surface (Figure 2Aviii) [20] and by using various sorbent materials (e.g., polydimethylsiloxane, hydrogel, filter paper, cotton pads, or polymer films) in a patch-type design (Figure 2Aix,xi,xii), which directly contact the skin [21], or are embedded in a solid support without direct contact with the skin (Figure 2Ax) [22]. For instance, the recently introduced **hydrogel micropatch** sampling (Figure 2Axii) [5,23–25] enables rapid collection of minute

identification of a biological system but is used instead as a screening tool to discriminate biological samples.

**NMR spectroscopy:** analytical technique that uses a strong external magnetic field and a weaker radiofrequency field to affect the behavior of the atomic nuclei within the molecule of sample, causing energy transfer at the wavelength that coincides with the radiofrequency. The signal that matches this transfer (energy gap) is then recorded using sensitive radio receivers.

**Pilocarpine:** cholinergic drug typically used to induce sweating by iontophoresis. It stimulates and binds to muscarinic receptors of eccrine sweat glands, which causes increased sweat production.

**Suction blistering:** technique for collecting skin interstitial fluid wherein a suction chamber maintained under vacuum is placed on the ventral forearm to induce blisters. The blisters are then punctured using a syringe to collect the interstitial fluid.

stressors, such as UV light (UVA and UVB) (F), as well as pollution in the form of gases and particulate matter contribute to the chemical composition of the outer layer of skin. Residues from topical drugs and consumer products (G) also affect chemical composition of skin. The human skin is home to diverse bacteria, fungi, and viruses comprising the skin microbiota. Thus, skin metabolite composition can be affected by the interactions of skin excretions as well as topical drugs and cosmetics with the skin microbiota (H).

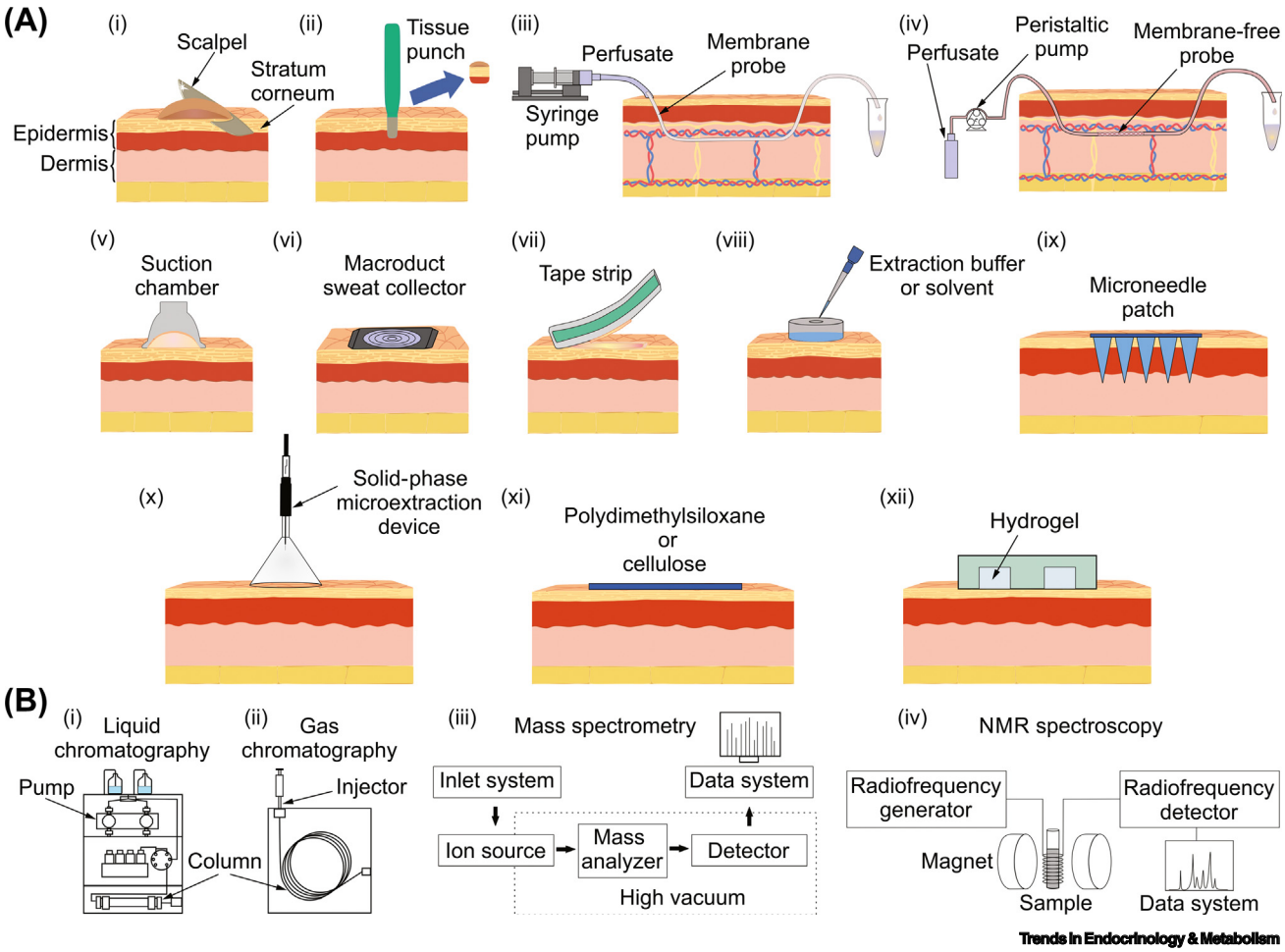


Figure 2. Analytical Methodology for Skin Sampling (A) and Metabolite Analysis (B); A Simplified Depiction of Representative Approaches. Skin-sampling protocols vary depending on the specific application in skin metabolomics. (A) Sampling protocols can be categorized as: invasive (i–iv), which can collect tissue specimen, interstitial fluid, epidermis and skin layers beneath the epidermis, and are performed in the clinic by medical experts; minimally invasive (v–ix) which can collect skin interstitial fluid, stratum corneum and skin excretions; and non-invasive (x–xii), which can collect skin excretions or emitted volatiles. (B) Analysis of skin metabolomics typically includes analytical platforms, such as chromatographic techniques (i,ii) coupled with mass spectrometry (iii), and NMR spectroscopy (iv).

amounts of skin excretions for MS analysis. Briefly, hydrogel (agarose) micropatches are created in cavities within a polytetrafluoroethylene plate. After specimen collection and transfer to the chemical laboratory, the probes are placed in the sample interface installed in the opening of a commercial mass spectrometer. The skin metabolites present in the hydrogel micropatches are re-extracted by a flowing ionization solvent. The extract is instantly transferred to the ion source of the mass spectrometer, and the ion signals are recorded. The recorded mass spectra are subjected to computational treatment, including normalization.

Some minimally invasive techniques that can sample skin metabolites have also been reported. For example, hydrogel microneedle patches were proposed as a minimally invasive technique to collect skin interstitial fluid (Figure 2Aix) [26]. The tape-stripping technique (Figure 2Avii) is another minimally invasive technique that samples the stratum corneum, wherein an adhesive tape is applied onto the skin surface and peeled off multiple times [14]. Before metabolite analyses, sample preparation often needs to be performed, which typically involves protocols such as

sample clean-up, extraction and analyte preconcentration. Sample extraction is done to transform the biological specimen into a more amenable state for analytical instrument detection [27].

Currently, the major detection platforms in metabolomics are **nuclear magnetic resonance (NMR) spectroscopy** and **MS**, in particular, MS techniques preceded by chromatographic separations (Figure 2B) [28]. NMR requires minimal sample preparation and, hence, is the preferred method for **metabolic fingerprinting** [29]. However, NMR sensitivity is lower than that of MS-based platforms. Therefore, hyphenated MS techniques [i.e., gas-chromatography-MS (GC-MS) and liquid chromatography-MS (LC-MS)] are used for comprehensive and sensitive metabolite identification and quantification [28]. Given the diverse metabolites involved, one analytical platform cannot cover the full skin metabolome. Thus, multiple analytical platforms are occasionally used simultaneously (e.g., GC-MS and LC-MS, NMR and LC-MS) [2,28].

The future of skin metabolomics as an efficient translational research tool depends on the development of convenient and reliable analytical workflows. Such workflows must involve analytical methodologies in sampling and sample preparation, detection, metabolomics data analysis, biological interpretation, and validation [2]. The analytical methods developed for metabolic studies require validation before their use in metabolite identification and quantitation. In method validation, challenges and limitations in each step of the metabolomics workflow are addressed.

Issues to address in skin metabolomics include interpersonal variability, low analyte concentrations, and the resulting difficulty in determining absolute concentrations over time, as well as the small amount of biological specimens collected [4]. Interpersonal (biological) variability of metabolite levels is a major concern in skin sampling. Skin is a 'dynamic' matrix; thus, metabolite levels change with time. The observed variability can even be caused by non-uniform distribution of chemical species on skin surface. Moreover, human skin is exposed to different stressors. Skin microbiota and the reaction of the skin to stressors vary from person to person, regardless of whether the individual is healthy or has a disease.

Another source of variability is the analytical process itself, which comprises sampling and detection. In skin sampling using hydrogel micropatches, Dutkiewicz *et al*. considered several approaches to normalize data to compensate for the variation in recorded analyte signals. These included addition of internal standards (i.e., isotopically labeled compounds) onto the collecting medium (hydrogel) and dividing extracted ion currents by partial total ion currents [24,25]. However, the method of normalization would normally depend on the specimen matrix, selected sampling method, detection method, and the specific objective of the analysis. In some methods targeting sweat, it is particularly challenging to compensate for the variations in the collected specimen volumes.

Overall, skin sampling and detection methods directly impact the quality of metabolomics data and the subsequent biological interpretation. Whether an application is for skin metabolite profiling, biomarker identification, or for targeted metabolite analysis, meaningful data sets, which would give sufficient molecular information to be clinically relevant, can only be attained by efficient sampling and detection techniques. By contrast, data analysis has the potential to be streamlined into a standardized approach because of the availability of specialized software tools (cf. [30]) and automation of data processing (cf. [31]).

## Relationship between Skin Metabolites and Blood Metabolites

Non-invasive sampling and analysis of skin metabolites can be viable tools in disease diagnosis and health monitoring. In addition to research focusing on identifying skin metabolite biomarkers

for disease, the development of minimally invasive and non-invasive devices as an alternative way to monitor health has been the focus of recent studies [6,32]. Biosensors for detecting the composition of alternative biofluids, such as sweat and interstitial fluid, are being developed with the objective to create point-of-care testing devices to complement and/or substitute standard assays [6,32]. Non-invasive sweat sensors, such as wearable sensors and epidermal sensors (i.e., for direct chemical sensing of the skin), have already been reported [32]. Wearable sensors include patch-like sensors attached to the skin and accessory-type sensors, such as watch and eyeglasses, while epidermal sensors include temporary tattoo-based electrochemical sensors [32]. Conversely, minimally invasive microneedle array sensors have been developed for detecting metabolites in the interstitial fluid of the skin [6]. Typical steps in biosensor fabrication include assembling electrodes on a substrate, incorporating a selective recognition element for the analyte, and utilizing a physicochemical transducer to transform any detected variations into a readable signal [32].

In the development of such sensors, the target analyte detected in sweat or interstitial fluid is evaluated for any correlation of the same analyte in blood. For example, sweat glucose and skin interstitial fluid glucose detected by non-invasive or minimally invasive sensors are compared with blood glucose measurements. Such a correlation would be particularly useful in developing alternative point-of-care testing to monitor the condition of patients with diabetes mellitus, for which glucose is an established biomarker. Moreover, evaluating and verifying the correlation between sweat or interstitial fluid and blood is influenced by intra- and interpersonal variability in the sampling protocol [6,33,34]. For example, glucose can be sampled from the stratum corneum, skin interstitial fluid, and sweat. Correlations were reported between sweat glucose and blood glucose because a rapid sweat response is collected quickly, reflecting glucose levels in the body [35]. Correlation between glucose in the interstitial fluid and in blood in steady-state conditions has also been reported [33]. In microneedle sensing, the skin sampling depth affects detection efficiency [6]. Intrapersonal variability due to glucose spatiotemporal dynamics among the layers of the skin (i.e., epidermis, dermis, and subcutaneous layer) and interpersonal variabilities, such as composition and thickness of these different layers, are factors that influence glucose detection in the interstitial fluid of the skin [34]. However, relationships between skin and blood metabolite levels have not been confirmed for most metabolites of clinical interest. Given that most findings on the human metabolome have been through blood-based detection [36], blood is considered the standard biofluid for monitoring physiological feedback [37]. Hence, further verification of skin and blood metabolite correlation is necessary to realize practicable applications of both non-invasive and minimally invasive skin metabolites analysis in the clinic.

## Translation of Skin Metabolomics to Clinical Applications

Metabolite biomarkers are discovered by profiling a particular metabolome, and are then validated through targeted analysis for absolute quantification [36]. Most biomarker discoveries are related to blood, tissue, and urine metabolome, and the metabolite biomarkers of clinical relevance are predominantly blood biomarkers (i.e., from serum or plasma) [36]. Conversely, skin metabolite biomarkers are not routinely used in most clinical diagnostics. The chemical composition of the skin matrix contains a huge amount of information regarding metabolic processes. Alterations to skin metabolites can be indicative of disease or other physiological states [9,25]. Moreover, skin metabolites reveal considerable spatial heterogeneity [38]. However, the lack of standardized methods and clinical validation studies on larger populations limits the potential of skin metabolite biomarkers for diagnostic applications. Nevertheless, skin metabolites still have potential as prospective biomarkers of diseases. Recent breakthroughs in the comprehensive analysis of metabolites in biological samples have stimulated research toward skin biomarker discovery [5]. Skin metabolic signatures could provide predictive, prognostic, diagnostic, and

surrogate markers reflecting diverse disease states, predicting the risk of acquisition, or revealing onset, progression, resolution, recurrence, and prognosis of specific diseases, as well as facilitating drug discovery/development (Table 1) [39].

### Skin Metabolites as Biomarkers of Skin Disease

Several reports have pointed to differences in metabolites within psoriatic skin lesions by comparing psoriatic lesions with symptom-free psoriatic and/or healthy controls, and suggested these metabolites as biomarkers of psoriatic disease (e.g., [25,40,41]). For example, a study conducted using hydrogel micropatch sampling and online MS detection revealed choline, glutamic acid, phenylalanine, lactic acid, urocanic acid, and citrulline as psoriasis-related metabolites [25]. Moreover, some metabolites correlated with disease severity, clinical improvement, and treatment response [25,40]. In another study, sweat derived from patients with atopic dermatitis had higher glucose concentrations than that from healthy subjects [42]. A skin metabolomics approach was used to identify characteristic biomarker profiles in several skin diseases, including melanoma [43], basal cell carcinoma [44], and atopic dermatitis [45].

### Skin Metabolites as Biomarkers of Systemic Disease

In addition to skin diseases, research is increasingly revealing the potential usefulness of skin metabolites as a source of biomarkers for a variety of systemic diseases [37]. For instance, sweat analysis has been routinely used to diagnose CF, – a severe autosomal recessive multisystem disease [46]. This disease is caused by mutations in the gene encoding CF transmembrane conductance regulator (CFTR). Such mutations lead to a dysfunctional CFTR protein in the sweat gland, causing alterations in the transport of sodium and chloride in epithelial-secreting cells as well as formation of mucus, which clogs the airways in the lungs. Most patients acquire a multisystemic disease involving lung, liver, and gastrointestinal

Table 1. Examples of Metabolic Disease Markers in Skin-Related Matrices

| Disease | Matrix | Skin biomarker | Remarks | Refs |
|---|---|---|---|---|
| CF | Sweat | Chloride | Simultaneous sweat tests/collection (quantitative pilocarpine iontophoresis test and Macroduct system) were performed on patients ($n$ = 1090; representing 63.8% of all sweat tests performed over a 10-year period) | [47] |
| Major depressive disorder in clinical remission | Sweat | Cytokines and neuropeptides | Women with major depressive order (mostly in clinical remission; $n$ = 20) and controls ($n$ = 19) wore skin patches for 24 h | [60] |
| Lung cancer | Sweat | Nonanedioic or azelaic acid, monoglyceride MG (22:2), suberic acid, a trihexose, and a tetrahexose | Prediction model built based on single panel of metabolites (five metabolites were combined) to discriminate patients with lung cancer ($n$ = 41) from a control group [without ($n$ = 31) and with risk factors (i.e., active smokers), ($n$ = 24)] | [61] |
| PD | Sebum | Volatile compounds (perillic aldehyde, hippuric acid, eicosane, and octadecanal) | Study performed on three stages: discovery ($n$ = 30), validation ($n$ = 31), and smell analysis by a human smeller ($n$ = 3) | [49] |
| Psoriasis | Skin excretions | Choline, citrulline, glutamic acid, urocanic acid, lactic acid, and phenylalanine | Patients with psoriasis ($n$ = 75 men and 25 women) and healthy controls ($n$ = 63 men and 37 women) matched for race, age, and gender<br>Additional healthy controls ($n$ = 11) participated in the study to assess method repeatability and reproducibility with respect to the six metabolites of interest | [25] |
| Schizophrenia | Skin lipids | Ceramides | Patients with a first episode of schizophrenia ($n$ = 22; 15 male and 7 female) and controls ($n$ = 22) matched for age and gender | [50] |
| Malaria | Skin volatiles | 4-Hydroxy-4-methylpentan-2-one, nonanal, toluene, and two unidentified compounds | Skin volatiles collected over a 4-year period from more than 400 primary-school children aged ≤12 years | [51] |

tract, as well as other organs in the body [46]. Patients with CF have high electrolyte concentrations in their sweat. Specifically, the sweat chloride level is disturbed due to CFTR dysfunction. Thus, sweat chloride is a biomarker used to diagnose CF [46,47].

In patients with severe uremia, high levels of urea are secreted by eccrine sweat glands as a component of sweat, which might be a biomarker for end-stage renal disease [48]. Recently, Trivedi *et al.* used thermal desorption-GC-MS alongside olfactory analysis to identify a distinct metabolic signature of PD [49]. According to the authors, the unique odor of patients with PD suggests an altered skin microflora and skin physiology. Comprehensive analysis of sebum collected from patients with PD showed a distinct volatome associated with their smell [49]. Several VOCs (perillic aldehyde, hippuric acid, eicosane, and octadecanal) were reported as potential biomarkers for non-invasive screening for PD. Furthermore, Smesny *et al.* investigated skin lipid metabolism in patients with antipsychotic-naive schizophrenia and healthy controls, and demonstrated sphingolipids as potential biomarkers of the disease [50].

Changes in the VOCs emanating from the skin are also reflected in infectious diseases, because the host odors may be altered by the pathogens causing the disease. Such is the case in the study by De Moraes *et al*. wherein a population with high rates of malaria was sampled for skin volatiles [51]. Their study revealed five predictors (4-hydroxy-4-methylpentan-2-one, nonanal, toluene, and two unidentified compounds) of malaria for both symptomatic and asymptomatic infections.

### Monitoring Therapeutic Effects of Administered Drugs

Various pharmacological metabolites, including 3,4-methylenedioxymethamphetamine [52], opioids [53], and tetrahydrocannabinol [54], have been detected in sweat excreted onto the skin surface. Moreover, Marchei *et al.* and Tsunoda *et al.* evaluated the excretion of therapeutic drugs and metabolites, such as atomoxetine and levodopa (L-DOPA), in patients with attention deficit/hyperactivity disorder [55] and PD [56], respectively, to monitor drug use, concentration, and metabolism.

### Drug Development for Skin Disease and Cosmetic Use

Several therapeutic drugs, consumer products, or cosmetics are topically applied to the skin, and research shows that the skin (via a variety of metabolic enzymes) has the ability to metabolize these agents [57], resulting in the transformation, degradation, or activation of these drugs, affecting their penetration, uptake, or elimination [58]. Traces of such products can be detected on skin using the techniques described earlier [24,59]. A better understanding of skin metabolism could assist safety assessment, and facilitate the development of new drugs for skin diseases or of cosmetic products [57].

## Concluding Remarks and Future Perspectives

Non-invasive skin sampling provides a simple approach for skin metabolome analysis, enabling dynamic and repetitive monitoring without patient discomfort. The protocols for non-invasive skin sampling are straightforward, and can be implemented after little training. Hence, non-invasive skin sampling could be used to sample a larger population. In doing so, more inclusive and unbiased results can be obtained for biological interpretation and validation in skin metabolomics studies. Further explorations need to be done to address the challenges that restrict the applications of skin metabolite analysis (see Outstanding Questions). In particular, further research is needed to understand the relationships between metabolites in blood and urine, physiological states, and skin metabolomes. If this goal is achieved, there will be a firm basis for the use of skin metabolic data in disease diagnostics and health monitoring.

### Outstanding Questions

Is there a quantitative relationship between metabolites in blood and skin? Studies of the correlation of blood and skin metabolites remain insufficient, in particular the lack of clinical studies involving larger populations to represent unbiased evaluation of such correlations.

Can robust technology be delivered to rapidly quantify multiple metabolites in clinical specimens? The ease of access for sampling skin metabolites provides an advantage in developing non-invasive and straightforward methodologies. By contrast, the skin is directly exposed to environmental stress and contamination, leading to variations in the collected specimens.

When implementing the available techniques of skin excretion sampling, can we distinguish between changes in the concentration of the sweat components and in the volume of the collected sweat?

Does the sampling process affect the composition of the collected skin excretions? Aside from interpersonal variability of skin excretions, intrapersonal variations exist for different skin-derived specimens (even for one individual). In the case of sweat, metabolite concentrations may vary for specimens obtained from different locations on skin alongside environmental conditions (temperature and humidity). In the case of interstitial fluid, metabolite concentrations may vary across the different layers of skin. When developing or choosing a sampling technique, these factors must be considered.

Can metabolite concentration reference ranges be defined for skin-related specimens with comparable confidence to those defined for blood and urine specimens? The concentration ranges of some metabolite biomarkers from blood and urine have been established, and are routinely used in clinical settings. Although standardized skin-sampling protocols are lacking, are current developments in non-invasive skin-sampling methodologies (and the advantages that these techniques deliver) suitable to establish skin metabolite concentration

reference ranges characterizing healthy populations?

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