Minireview

Towards standardized and reproducible research in skin microbiomes

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Summary

Skin is a complex organ serving a critical role as a barrier and mediator of interactions between the human body and its environment. Recent studies have uncovered how resident microbial communities play a significant role in maintaining the normal healthy function of the skin and the immune system. In turn, numerous host-associated and environmental factors influence these communities’ composition and diversity across the cutaneous surface. In addition, specific compositional changes in skin microbiota have also been connected to the development of several chronic diseases. The current era of microbiome research is characterized by its reliance on large data sets of nucleotide sequences produced with high-throughput sequencing of sample-extracted DNA. These approaches have yielded new insights into many previously uncharacterized microbial communities. Application of standardized practices in the study of skin microbial communities could help us understand their complex structures, functional capacities, and health associations and increase the reproducibility of the research. Here, we overview the current research in human skin microbiomes and outline challenges specific to their study. Furthermore, we provide perspectives on recent advances in methods, analytical tools and applications of skin microbiomes in medicine and forensics.

Introduction

Human skin is a complex organ that plays a diverse role in human health and protects us from various environmental exposures (Uberoi et al., 2021). Skin maintains balance and homeostasis by forming an active barrier between the internal organs and the external environment (Sotiropoulou and Blanpain, 2012). It provides a habitat for a diverse ecosystem mainly consisting of beneficial (Christensen and Brüggemann, 2014; Delanghe et al., 2021; Fang et al., 2021) and potentially pathogenic microbes (Findley and Grice, 2014; Hwang et al., 2021). As a growth environment, human skin is primarily dry, acidic, nutrient-poor and cool (Grice and Segre, 2011). In healthy humans, skin temperature averages between 33°C and 34°C indoors, reaching down to 19°C or up to 35°C when exposed to outdoor temperatures between 0°C and 35°C (Lai et al., 2017). Even temperatures close to 37°C can be reached in the groin and armpits (Boxberger et al., 2021). Thus, the skin is highly selective on the types of bacteria, archaea and eukaryotes that survive and thrive on it (Grice and Segre, 2011; Byrd et al., 2018). Stable communities of commensal microbes are primarily established on skin surfaces during the first weeks of a newborn’s life, with specific types of communities developing at different body sites (Luna, 2020). However, the communities continue to mature during their whole life, and persisting microbes can be introduced to the community through repeated invasion and colonization until adulthood (Grice and Segre, 2011; Swaney and Kalan, 2021). Through the combination of factors specific to the human skin as an environment, microbial communities on it are unique and distinct from the skin microbiomes of other mammals (Ross et al.,...
Overall, the human skin microbiome represents a complex and dynamic system with significant contributions to the health and development of various diseases and disorders (Byrd et al., 2018; Swaney and Kalan, 2021).

**Microbial community assembly on the skin**

Like other biological communities, cutaneous microbiota is governed by the constant ecological processes of dispersal, selection, drift and diversification (Kennedy and Chang, 2020). Major phyla of bacteria found on human skin are *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Bacteroidetes*, although their relative abundances vary between persons, throughout their lifespan (Luna, 2020), and between body sites on an individual (Byrd et al., 2018).

Correspondingly, the relative abundances of archaea are usually scarce and differ from childhood to old age, but most belong to either phyla *Thaumarchaeota* or *Euryarchaeota* (Moissl-Eichinger et al., 2017; Umbach et al., 2021). In addition, fungi, such as genera *Aspergillus*, *Penicillium*, *Candida* and *Cryptococcus*, are commonly found on healthy skin (Leung et al., 2016). Species from the genus *Malassezia* are the most commonly occurring fungi on most skin sites and are usually considered part of the healthy skin microbiota (de Hoog et al., 2013). The initial composition with other members of the microbiota have been only rarely studied (Hannigan et al., 2015; Liang and Bushman, 2021).

Many eukaryotic viruses from families *Polyomaviridae*, *Papillomaviridae*, *Circoviridae*, *Caudovirales Adenoviridae*, *Anelloviridae* and *Herpesviridae* are commonly detected (Foulongne et al., 2012; Hannigan et al., 2015). The interactions of the virome with other members of the microbiota have been studied (Hannigan et al., 2015; Liang and Bushman, 2021). However, the skin virome can also contribute to the health and disease of the host through the suppressive actions of bacteriophages (Wang et al., 2020).

Most of these overarching patterns have been observed when sampling adult subjects. However, the human skin microbiome also changes considerably over time. While microbes have been reliably detected in the human womb (Fricke and Ravel, 2021), the existence of a stable microbiome in the foetal environment before delivery seems unlikely (for a recent discussion on this subject, see Blaser et al., 2021). The initial composition of the skin microbiome is thus primarily affected by maternal factors, such as birth mode, which lead to the dispersal of different organisms on the skin (Bokulich et al., 2016; Stennett et al., 2020). This initial assemblage of microbes is based on different microenvironments of the skin, which vary physicochemically and topographically due to differences in pH, dryness, thickness, folding, the density of hair follicles and glands, and exposure to the environment (Grice and Segre, 2011; Cho and Eom, 2021; Sun and Rieder, 2021). The different layers of skin, from the epidermis to the dermis, and even the adipose tissue, are colonized with unique subsets of microbes in the separate cutaneous compartments (Zeeuwen et al., 2012; Nakatsuji et al., 2013). This deep penetration of the skin by certain bacteria can lead to immediate immune responses in the host (Nakatsuji et al., 2016). In addition, the initial skin microbiome influences the developing immune system and may even affect the person’s susceptibility to diseases later in life (Neu and Rushing, 2011; Bokulich et al., 2016; Dunn et al., 2017). After birth, bacterial diversity of the skin has been observed to increase with age, at least until the pre-puberty stage (Lehtimäki et al., 2017; Zhu et al., 2019). During the maturation process, the initially dominant bacteria *Lactobacillales* (especially genus *Streptococcus*) are replaced by the members of both *Actinobacteria* (genera *Propionibacterium* and *Corynebacterium*) and *Proteobacteria* (Lehtimäki et al., 2017). Furthermore, the fungal microbiome of the skin appears to be highly unstable and diverse at birth, but its stability increases, and diversity decreases within the first 6 months (Zhu et al., 2020). Fungal diversity decreases until puberty and converges into a more stable community dominated by *Malassezia* (Jo et al., 2016). The species composition then shifts to obligatorily lipophilic fungi by adulthood (Jo et al., 2016; Leung et al., 2016; Zhu et al., 2020).

The overall maturation and development of the microbial communities during childhood affects all skin sites or compartments, but the community compositions at individual skin sites also differ considerably. Many microbial species can modify the skin environment through their metabolism, such as *Cutibacterium acne*, which can break down the sebum lipids into fatty acids and acidify the skin (Flowers and Grice, 2020). The features of the skin site considerably affect its bacterial composition (Byrd et al., 2018). Skin sites can roughly be categorized into dry, sebaceous and moist sites (Fig. 1; Byrd et al., 2018). The volar forearm is a commonly sampled dry skin site which was used in a number of pioneering studies (Gao et al., 2007; Grice et al., 2009; Kong et al., 2012; Findley et al., 2013), likely due to its easy accessibility. These studies have provided convenient reference points for current skin research (e.g. Bjerré et al., 2019). For sebaceous sites, the forehead has been similarly easy to sample, and it is an affected area in several inflammatory skin diseases (Dekio et al., 2005; Alexis and Talbott, 2021). For moist sites, the bend of the elbow is one of the most often sampled sites, likely also because of its easy accessibility and involvement with, e.g. atopic dermatitis in children (Kong et al., 2012; Byrd et al., 2018; Alexis and Talbott, 2021).
Generally, dry sites have a high prevalence of genera *Propionibacterium*, *Staphylococcus* and *Corynebacterium*, sebaceous sites are dominated by *Propionibacterium* and *Staphylococcus*, and moist sites have high abundances of *Corynebacterium* and *Staphylococcus* (Byrd et al., 2018). While relatively little is known about similar site-specificity of archaea, their abundance appears to be higher on dryer skin with lower sebum levels and lipid content (Moissl-Eichinger et al., 2017). In the fungal microbiome of the skin, *Malassezia* species dominate throughout the body, except for the feet, where fungal diversity is very high (Grice and Dawson, 2017). However, individual *Malassezia* species can display considerable site-specificity. *M. restricta* is predominant on facial surfaces, and *M. globosa* is prevalent in the back (Grice and Dawson, 2017). Biological interactions among microbes and between the host and microbes are crucial for the development and composition of the community. For example, *Staphylococcus aureus* colonization on the skin is prevented by antibacterial compounds produced by *S. lugdunensis* (Zipperer et al., 2016) and through antimicrobial peptide production of host keratinocytes induced by *S. epidermidis* (Iwase et al., 2010). These complex interspecies interactions likely play a role in their uneven distribution in the different microenvironments on the skin (Hernandez-Valdes et al., 2020).

**Inter-individual differences in skin microbiome composition**

In addition to these generic patterns, there is considerable inter-individual variability in skin microbiome composition. This variability originates from intrinsic factors, including phenotypic differences between hosts, and external factors, such as environmental exposures (Table 1). Many intrinsic factors, such as genetics, sex, ethnicity and age, have been connected with changes in the skin microbiome composition (Si et al., 2015; Ying et al., 2015; Li et al., 2019; Carrieri et al., 2021). For example, specific bacterial taxa, such as *Roseomonas* and *Corynebacterium*, likely have high heritability (Si et al., 2015). Sex and ethnicity also correlate with differences in bacterial community structure on comparable skin sites (Li et al., 2019). Furthermore, the skin microbiome compositions in a small ethnically homogeneous cohort of healthy women could be used to accurately model and predict skin hydration, age, smoking and menopausal status (Carrieri et al., 2021). However, the role of

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**Fig. 1.** Categorization of common sampling sites for skin microbiomes. The most abundant groups of microbes in each category of sites are shown.
interconnected variation in, e.g. skin pH, sebum or sweat production, hormone levels and cosmetics application frequency cannot be ruled out as causes for some of the observed intrinsic variation (Fierer et al., 2008; Ying et al., 2015). In addition to intrinsic variation, multiple external factors alter the skin microbiota composition (Table 1; Moskovicz et al., 2020). These effects have been attributed to, for example, contact with other people and dogs (Song et al., 2013), exposure to environmental microbes (Nielsen and Jiang, 2019; Vandegrift et al., 2019), and environmental conditions such as UV radiation (Burns et al., 2019). Also, lifestyle-related variables, such as diet (Brandwein et al., 2019), medications (such as antibiotics; Park et al., 2020), cosmetics (Bouslimani et al., 2019) and contact with housing materials or chemicals (McCall et al., 2020), are known to influence the skin microbiome. The effects of external factors can be either temporal or long-lasting. For example, the residence time of introduced microbes can be affected by the quantity of biomass of the source and the duration of the interactions with it (Vandegrift et al., 2019). Healthy skin communities display some resilience to such disturbances and can maintain a stable composition over long periods (Oh et al., 2016; Moskovicz et al., 2020; Hillebrand et al., 2021). However, high frequency or constant interaction with specific external factors can likely lead to more permanent changes in the skin microbiome. As many environmental factors which can affect the skin microbiome have uneven geographical distributions, the place of residence of an individual is also reflected in their skin microbiome composition (Callewaert et al., 2020). For example, compositional differences have been observed between urban and rural individuals (Ying et al., 2015; Lehtimäki et al., 2017), and even between residents of different cities (Sun et al., 2019). The compositions of the site-specific skin microbiomes on a person are dependent on both internal and external factors and their varying effects over that person’s lifetime. Therefore, skin microbiomes are highly unique and may even be used to identify an individual (Fierer et al., 2010; Schmedes et al., 2017). For example, only 2% of species-level operational taxonomic units (OTUs) were found to be shared among individuals on superficial forearm skin in a study published over a decade ago (Gao et al., 2007). Since this seminal study, several promising forensic applications have been identified for skin microbiomes, such as identifying people, their gender or geographical origin, or which individual or body site likely interacted with an object (Neckovic et al., 2020). However, a number of

### Table 1. Reported intrinsic and extrinsic factors influencing microbiome composition at various body sites.

| Factor                          | Intrinsic (I) or extrinsic (E) | Reportedly affected/sampled skin sites                              | Associated taxa                                                                 | References                                                                 |
|---------------------------------|--------------------------------|--------------------------------------------------------------------|--------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Genetics                        | I                              | Inner wrists                                                      | Brevibacterium, Corynebacterium, Peptoniphilus, Roseomonas                    | Si et al. (2015)                                                          |
| Ethnicity                       | I                              | Multiple body sites                                               | Corynebacterium, Proteobacteria, Staphylococcus                               | Li et al. (2019); Wang et al. (2021)                                       |
| Sex (F)                         | I                              | Multiple body sites                                               | Corynebacterium, Propionibacterium, bacterial diversity                      | Fierer et al. (2008); Ying et al. (2015); Li et al. (2019)                 |
| Age                             | I                              | Multiple body sites                                               | Corynebacterium, bacterial diversity, archaeal diversity, fungal diversity    | Ying et al. (2015); Jo et al. (2016); Lehtimäki et al. (2017); Moissl-Eichinger et al. (2017); Li et al. (2019); Zhu et al. (2019, 2020) |
| Contact with people             | E                              | Palms                                                            | Propionibacterium, oral tax                                                                 | Song et al. (2013)                                                          |
| Contact with dogs               | E                              | Palms                                                            | Methylophilaceae, Actinobacteria                                                | Song et al. (2013)                                                          |
| Contact with environmental microbes | E                            | Exposed sites                                                     | Depending on the environment                                                  | Ying et al. (2015); Lehtimäki et al. (2017); Nielsen and Jiang (2019); Vandegrift et al. (2019) |
| Environmental conditions (UVA and UVB) | E                            | Exposed sites                                                     | Cyanobacteria, Lactobacillaceae, Pseudomonaceae                               | Burns et al. (2019)                                                        |
| Geography                       | E                              | Multiple body sites                                               | Depending on location                                                          | Sun et al. (2019); Callewaert et al. (2020); McCall et al. (2020) Park et al. (2020) |
| Medications (antibiotics)       | E                              | Cheek                                                            | Cutibacterium acnes, Cutibacterium granulosum, bacterial diversity            | Bouslimani et al. (2019)                                                   |
| Cosmetics                       | E                              | Armpits, feet                                                    | Depending on body site, sex and compound                                      |                                                                           |
| Diet (BMI)                      | E                              | Multiple body sites                                               | Corynebacterium, bacterial diversity                                           | Brandwein et al. (2019)                                                   |

*The effects of genetics and ethnicity can be largely overlapping.*

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challenges remain before skin microbiomes can be routinely used in forensics. For example, sampling and sample handling need to be standardized, reference databases have to be improved, and issues with contamination and legislation need to be resolved (García et al., 2020; Neckovic et al., 2020).

Skin microbiomes in health and disease

Humans have coevolved with their skin microbiota (Ross et al., 2018). Thus, the communities found on healthy skin generally support the function of the skin as a barrier and prevent the growth of harmful microbes on it (Byrd et al., 2018). In addition, skin bacteria seem to play a major role in training our adaptive immune system (Naik et al., 2012). This process occurs during childhood and depends on exposure to diverse environmental microbes in the person’s living environment (Lehtimäki et al., 2017). The combined effects of the whole community on skin function appear to be more than a sum of its parts. For example, the regulatory effects of the whole microbial community were more extensive and pronounced than those of individual microbes in a 3D human skin model (Loomis et al., 2021).

As the biodiversity of our living environment is reflected in the biodiversity on our skin, changing exposures of the skin microbiome have predisposed people in industrialized environments to both cutaneous and inflammatory non-communicable diseases (Prescott et al., 2017). Globally, the most common skin diseases associated with microbes, in order of disease-adjusted life years, are atopic or seborrheic dermatitis, acne vulgaris, urticaria (associated with gut microbiome), psoriasis, viral diseases, fungal diseases, scabies, pyoderma, cellulitis and decubitus ulcer (Table 2; Karimkhani et al., 2017; Nabizadeh et al., 2017; Ellis et al., 2019; Pang et al., 2019). Notably, many of these diseases, such as psoriasis and atopic dermatitis, are not directly caused by a single pathogen but are in addition connected to microbiome immune system imbalances, dysbiosis (Kong et al., 2012; Catinean et al., 2019), or result from complex microbial interactions affecting the prevalence of virulence genes (Barnard et al., 2016). Even diseases caused by single pathogens, such as fungal and viral infections, might involve interactions with other microbes on the skin and the host immune system. For example, commensal Malassezia species usually display pathogenic features and cause infections (Grice and Dawson, 2017). The pathogenicity of Staphylococcus epidermidis, which is ubiquitous on healthy human skin, is dependent both on the specific strain and the biological context (Brown and Horswill, 2020). Also, skin microbiome dysbiosis has been associated with infections caused by eukaryotic parasites, such as human itch mite (Sarcoptes scabiei var. hominis; Bhat et al., 2017) and Leishmania species (Gimblet et al., 2017).

The interactions related to skin microbiomes, including environmental exposures and the host immune system, strongly influence the host’s overall health (Prescott et al., 2017). Unfortunately, mechanistic understanding of these systems has remained incomplete. Advances in sampling, analytical and algorithmic methods currently enable the examination of skin microbiomes at an unprecedented depth (Carrière et al., 2021; Loomis et al., 2021). The availability of high-throughput sequencing (HTS), together with efficient algorithms and computational resources to analyse the massive data sets, has revolutionized our view of the skin microbiome (Liu et al., 2021). However, there are several essential considerations in the current methods, which can improve the reliability and reproducibility of the data and analytical results. Furthermore, emerging technologies, such as long-read sequencing (Amarasinghe et al., 2020) and single-cell genomics (Sharma and Thaiss, 2020), will improve our understanding of these systems. Their application could further develop novel medical diagnostic tools and treatments, lessening the global burden of skin diseases.

Current analytical approaches

Study design

In skin microbiome research, data collection often starts with recruitment of suitable participants and choosing the skin site for sampling (Fig. 2). The exact criteria for choosing participants and their measured medical or demographic variables are highly context-dependent. However, factors affecting the skin microbiome, in general, should be considered in the study design. For example, the ethnic and geographic origin of the participants, and their age distribution, can influence the results and their generalization from individuals to populations and between different populations (Gupta et al., 2017). Statistical power, i.e. the number of participants needed to detect significant differences between the groups of interest, should preferably also be estimated prior to gathering data (La Rosa et al., 2012; Kong et al., 2017). The exact number of required samples for sufficient power depends on the probability model parameters, the effect size, presence of overdispersion in the data and even the number of reads per sample in sequencing studies (La Rosa et al., 2012). While recommendations for the exact number of participants and samples cannot be given, landmark studies in skin microbiomes have usually analysed dozens (e.g. Kong et al., 2012) to several hundreds of samples (e.g. Song et al., 2013). A number of power estimation tools have also been developed, such as...
Table 2. Associations between common skin diseases and skin microbiota.

| Skin disease       | Typical body sites                                                                 | Positive associations (relative abundance | Negative associations (relative abundance | References                      |
|--------------------|-------------------------------------------------------------------------------------|---------------------------------------------|---------------------------------------------|----------------------------------|
| Atopic dermatitis  | Bends of elbows and knees                                                           | *Staphylococcus aureus*,                   | *Malassezia*, bacterial                     | Kong et al. (2012); Chng          |
|                    |                                                                                    | *(Staphylococcus epidermidis in less severe AD)* | diversity, eukaryotic                      | et al. (2016); Sun               |
| Acne vulgaris      | Sebum-rich areas (e.g. scalp, face, shoulders)                                      | *Cutibacterium acnes*,                     | *Staphylococcus epidermidis*                | Barnard et al. (2016); Skabytska  |
|                    |                                                                                    | *Cutibacterium granulosum*                 |                                             | and Biedermann (2016); Park et     |
| Psoriasis          | Scalp, elbows, knees, lower back                                                    | *Corynebacterium, Propionibacterium*,      | *Cupriavidus*, *Flavisolibacter*, *Lactobacillus*, *Methylbacterium*, *Schlegella*, | et al. (2013); Wang               |
|                    |                                                                                    | *Staphylococcus*, *Streptococcus*,         | bacterial diversity                         |                                 |
|                    |                                                                                    | *Thermomonas*, pathogenic                   |                                             |                                  |
|                    |                                                                                    | *(e.g. Vibri)*                              |                                             |                                  |
| Scabies            | Multiple sites                                                                      | *Staphylococcus aureus*,                   | Not reported                                | Whitehall et al. (2013); Bhat et  |
|                    |                                                                                    | *Streptococcus pyogenes*                   |                                             | (2017)                           |
| Cellulitis         | Disrupted sites (e.g. bites, cuts, wounds, dry skin)                               | *Enterobacter, Enterococcus*,              | Not reported                                | Doern et al. (1999); Johnson et   |
|                    |                                                                                    | *Escherichia coli*, *Pseudomonas aeruginosa*, *Rhodanobacter terrae*, *Staphylococcus aureus*, *Streptococcus pyogenes* |                                             | (2016)                           |
| Decubitus ulcer    | Skin over bony areas (e.g. sacrum, heels, elbows, hips)                             | *Christensenella, Enterococcus*,           | *Actinobaculum*, *Mycobacterium vaccae*     | Wolcott et al. (2016); de         |
|                    |                                                                                    | *Eubacterium dolichum*, *Lactobacillus zeae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* |                                             | Wert et al. (2020)               |
| Leishmania infection | Exposed parts of the body                                                            | *Staphylococcus, Streptococcus*             | *Bacterial diversity*                       | Gimblet et al. (2017)            |

micropower for differences in β-diversity (Kelly et al., 2015), and HMP for differences in relative abundances (La Rosa et al., 2012). Insufficiently small sample size is also a current problem in human microbiome studies utilizing machine learning, as it can lead to a biased estimate of a model’s predictive performance (Quinn, 2021). After deciding on the study design, necessary measurements based on its specific demands are then collected from the participants. These will further depend on the project goals and limitations of the available equipment or other resources (Jarett et al., 2021).

Sample collection

In the cutaneous sampling, the site and technique choice greatly affect which organisms are enumerated in the sample (Fig. 2; Prast-Nielsen et al., 2019; Bay et al., 2020). Because of the high number of external factors that can affect the skin microbiome’s composition, exposures of the sampling site to such factors should be controlled before sample collection. For example, participants have been instructed to avoid washing and the use of skin emollients before sampling, often for 12–24 h, and those using systemic or topical antimicrobial compounds have usually been excluded (Kong et al., 2017). Both handwashing with soap (Two et al., 2016) and swimming in the ocean (Nielsen and Jiang, 2019) can alter the skin microbiome at least for 24 h. Thus, participants should be instructed to avoid washing, bathing and swimming for at least 24 h. While effects of washing might persist over longer times, longer time limits would likely be impractical for recruitment of participants. However, the use of antibacterial soap affects the skin microbiome at least for 2 weeks (Yu et al., 2018). The use of any antibacterial compounds in soaps and emollients should therefore be more carefully controlled, and participants using such products should also be excluded. Avoiding skin washing also has the added benefit of somewhat increasing the naturally low biomass of the skin microbiome (Bjerré et al., 2019). Samples with low microbial biomass are, for example, highly prone to DNA contamination from external sources. Methods to control and account for such contamination have been suggested, such as randomizing sample types and treatment groups during sample collection (Eisenhofer et al., 2019).

In most studies, the body site for sampling has been defined as a distinct anatomical location, such as the volar forearm or forehead (Fig. 1). However, care should be taken when defining the exact sampling site, as, e.g. handedness (which hand is dominant) can lead to
differences in microbiota composition at an anatomically identical sampling site (Lehtimäki et al., 2017). The conditions within a specified body site might also vary with corresponding small-scale differences in the microbiome (Bjerre et al., 2019). This type of high-grain spatial organization of the microbiome might explain some of the sampling bias observed with the most common sampling methods (Prast-Nielsen et al., 2019). Micrometre-scale spatial organization of bacteria on tongue dorsum has been recently described using multiplexed fluorescence spectral imaging (Wilbert et al., 2020). Similar approaches applied to skin microbiomes could likely elucidate these poorly known high-grain spatial patterns. For example, a recent study on spatial organization of C. acnes on facial skin revealed that individual pores are dominated by single lineages, enabling the stable coexistence of diverse strains of the bacterium (Conwill et al., 2022).

Currently, the most common sampling methods for skin microbiomes are swabbing, tape-stripping, scraping and punch biopsy (Fig. 2; Bjerre et al., 2019; Prast-Nielsen et al., 2019). These techniques differ, for example, in their invasiveness and discomfort level, depth of skin sampled, amount of biomass recovered and sampling bias (Table 3). Recent comparisons between these methods for HTS studies exist, e.g. for swabbing, tape stripping and scraping (Chng et al., 2016), swabbing and tape stripping (Ogai et al., 2018), swabbing and scraping (Bjerre et al., 2019), and swabbing and biopsies (Prast-Nielsen et al., 2019). Substantial differences can also exist within these methods. For example, the material of the swab (Warnke et al., 2014) and adhesive tape (Ogai et al., 2021) have measurable effects on the recovery of skin microbes. Overall, most sampling methods produce similar results for the abundant taxa, but differences can be significant for rare low abundance taxa. Thus, both the sampling area and the technique to be used are essential questions and should be weighted accordingly. For example, either tape stripping or punch biopsy should be used in studies where fine-scale spatial patterns on the skin need to be examined, as they technically enable the preservation of spatial patterns of the skin.
microbiome (Table 3). Several sampling methods can be used simultaneously, but this will lead to higher sampling effort and costs. More intensive sampling can also increase the participants’ discomfort. However, this can be justified, e.g., for the use of both swabbing and biopsies when studying diseases in the dermis (Prast-Nielsen et al., 2019). Importantly, consistent sampling of a well-defined site with similar sampling methods needs to be applied to ensure the replicability and comparability of the results. Here, standardization of the size and position of the swabbed area or tape strip could likely be used to lower sampling bias, at least within a single study. Factors which could help with standardization are, for example, uniform sampling equipment (same size and manufacturer), reporting of the exact size of the sampling area (such as 5 x 5 cm) and location (e.g., volar forearm on non-dominant side), and accounting for interpersonal differences (e.g., applied pressure, time and swiping pattern). While some of these factors can be assessed in data analysis, further research is still needed to develop sampling tools and methods with reduced bias.

**Sample processing**

After the sample collection, several routes can be taken to examine the microbial community in the samples. Notably, all processing immediately after sampling should consider sample exposure time to different ambient temperatures to minimize variation between the samples (Fig. 2). Storage temperatures have a limited effect on the microbial diversity for molecular analyses (Lauber et al., 2016). Cryogenic storage of tissue samples in liquid nitrogen (−196°C) can accurately preserve DNA and RNA for over a decade (Kelly et al., 2019), but we are not aware of similar studies on skin microbiomes, likely due to the brief history of the field. While further research is still needed, increasingly low temperatures are probably better also at preserving all types of skin microbiome samples (biopsies, swabs and strips) as long as the device materials tolerate this.

In addition to storage temperatures, the use of stabilizing agents such as RNA Later™ and DNA/RNA Shield™ can introduce bias to the analyses (Menke et al., 2017; Angebault et al., 2018; Hallmaier-Wacker et al., 2018). A recent comparison of storage methods applicable to resource-limited settings found that their effect on the differences in microbiota composition was lower than that of the collection method or site (Manus et al., 2022). However, the low biomass of skin swab samples also appears to make them more susceptible to disruption during storage than faecal and saliva samples (Marotz et al., 2021). Best practices might also depend on practical considerations, such as cost-effectiveness and availability of low-temperature storage (Marotz et al., 2021). Thus, sample storage should be carefully evaluated based on current research, and the use of control samples undergoing similar treatment as the skin samples is strongly recommended to minimize possible bias.

Currently, most skin microbiome studies utilize solely culture-independent methods based on high-throughput sequencing instead of isolating and culturing single organisms from the samples (Fig. 2). For this purpose, nucleic acids (DNA or RNA), proteins, or metabolites are extracted from the samples and analysed. The choice of the extraction method can significantly affect the downstream analyses. For example, notable differences exist between commercial DNA extraction kits (Bjerve et al., 2019). In addition, contamination within the extraction

| Method          | Description                                                                                           | Depth sampled | Invasive | Requires expert user | Amount of biomass | Pros                                                                                       | Cons                                                                                                                                       |
|-----------------|-------------------------------------------------------------------------------------------------------|----------------|----------|----------------------|-------------------|--------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| Swabbing        | Skin is swabbed with a sterile (wetted) fibre-tipped swab                                             | Epidermis      | No       | No                   | +                 | Widely used method increases comparability                                                  | Only samples the top layer of skin                                                                                                          |
| Tape stripping  | Sterile adhesive tape is attached to the skin and removed                                             | Epidermis      | No       | No                   | +                 | Preserves spatial structure in two dimensions                                                 | Can be unsuitable for fragile skin                                                                                                        |
| Scrapping       | A cup or scalpel is used to scrape the skin to obtain a sample                                        | Epidermis      | No       | No                   | ++                | Samples slightly deeper than swabbing and tape stripping                                   | Can be unsuitable for fragile skin. High number of human cells in samples                                                                 |
| Punch biopsy    | A small piece of skin tissue is removed with a sharp, circular and hollow instrument                  | Epidermis and dermis | Yes      | Yes                  | +++               | Preserves spatial structure in three dimensions                                               | Not well-suited for exposed skin surfaces. Very high number of human cells in samples                                                   |
kits, laboratory reagents, equipment and also cross-contamination from other samples and sequencing runs can be an issue in molecular analyses of low biomass samples, such as the skin microbiome (Salter et al., 2014; Kim et al., 2017; Eisenhofer et al., 2019). Therefore, proper negative and positive controls with defined microbial composition should be included throughout sample processing to reveal possible biases and account for their effects (Hornung et al., 2019). Specific guidelines for conducting and reporting low biomass microbiome studies have also been recently developed (Eisenhofer et al., 2019). These guidelines include, e.g. the use of clean environments and protective clothing, and decontaminating consumables, containers and reagents (for further discussion, see Eisenhofer et al., 2019). In skin microbiomes, the amount of host DNA can be upwards of 90% of total extracted DNA, which can be challenging for downstream analyses, as a large part of the sequencing effort in shotgun metagenomic sequencing is spent on the human genome instead of the microbiome (Bjerre et al., 2019). A customized chemical extraction method with propidium monoazide decreased human DNA reads by an order of magnitude and outperformed several commercial kits with a low taxonomic bias when analysing saliva samples (Marotz et al., 2018). A recent study used a commercial kit for host DNA depletion to reduce human reads from 92% to 32% in skin samples, but the taxonomic bias and problems with library construction were deemed too high for practical applications (Ahannach et al., 2021a). In the same study, propidium monoazide and the two commercial kits appeared to also decrease the relative abundances of Gram-negative microbes in 16S rRNA gene amplicon sequencing of skin samples. Thus, host DNA depletion cannot be currently recommended for skin microbiome research because the observed taxonomic bias cannot be justified purely by decreased sequencing costs.

**Culture-based approaches**

Culturing selects microbes best adapted to the utilized growth conditions, such as nutrient availability, pH and temperature (Bonnet et al., 2020). This focuses culture-based analyses on the cultivable minority of skin microbes. For example, culturing detected only 16% of the OTUs found with 16S rRNA sequencing in chronic wound infections, while sequencing detected 85% of the cultured strains (Rhoads et al., 2012). Overall, culture-independent methods enable a more detailed characterization of the skin microbiome than culture-based methods (Table 4; Timm et al., 2020) and should thus be used for diversity analyses. However, culturing provides an unparalleled opportunity to study an individual microbial strain’s growth, metabolism and biological interactions in controlled conditions (Thrash, 2019). Standardized culture-dependent methods are also often used in clinical microbiology (Boers et al., 2019). For example, the susceptibility of microbial strains to antibiotics is difficult to estimate with culture-independent methods (Vasala et al., 2020). Culture-based studies also widely benefit culture-independent studies. For example, culturing enables the identification of new species for improving reference databases (Nasko et al., 2018). Cultured species and strains can also be deposited in culture collections, enabling the in-depth study of microbes detected with culture-independent methods (Timm et al., 2020). Furthermore, culturomics has emerged as a promising high-throughput method to identify and characterize novel microbial species while simultaneously obtaining them in culture (Lagier et al., 2018). This approach consists of inoculating samples into varying conditions to obtain even tens of thousands of individual bacterial colonies, rapidly and cheaply identifying known species with matrix-assisted laser desorption ionization time-of-flight mass spectrometry and characterizing the unidentified species with 16S rRNA amplicon sequencing. This method has also been successfully applied on human skin for bacteria (Cassir et al., 2015) and fungi (Leong et al., 2021).

**High-throughput sequencing**

HTS has revolutionized our understanding of the microbial world (Blaser, 2014; Kanangat and Skaljic, 2021; Ahannach et al., 2021b), as it has enabled the detection and characterization of the difficult-to-culture majority of microbes in a wide range of environments, including the human body (Boers et al., 2019). Culture-independent methods such as amplicon sequencing and shotgun metagenomics are currently utilized in most skin microbiome studies (Fig. 2; Kong et al., 2017). Amplicon sequencing is most commonly used to profile the archaeal and bacterial communities. Here, a section covering a few hundred nucleotides of the highly conserved 16S ribosomal RNA gene is amplified and sequenced with HTS (Klindworth et al., 2013). Recently, amplicon sequencing of the nuclear ribosomal internal transcribed spacer (ITS) region has also been used to obtain (sub)species resolution of bacterial communities (Milani et al., 2020). ITS-based profiling might thus serve as a viable alternative for 16S rRNA-based profiling of bacterial communities in the future. For fungi, various targets in the ITS or small and large subunit ribosomal RNA genes (SSU/LSU) are used (Nilsson et al., 2019). As a cost-effective and relatively simple method, amplicon sequencing (i.e. metabarcoding) has been widely used for characterizing the microbiomes’ taxonomy and diversities. The functional potential of the community can also

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Table 4. Comparison of several culture-based and culture-independent approaches for analysing skin microbiomes.

| Category         | Method                      | Costs per sample | Coverage for taxonomic characterization | Functional analysis | Characterization of novel microbes | Identification of known microbes | Relevant applications | Other pros | Other cons | References                                      |
|------------------|-----------------------------|------------------|----------------------------------------|---------------------|-----------------------------------|---------------------------------|------------------------|------------|-----------|------------------------------------------------|
| Culture-based    | Isolation of pure cultures  | +++              | X X X NA ++                           | X                   | Mechanistic studies               | Well-established standard methods | Organisms can grow very slowly or require complex or unknown growth media | Thrash (2019); Timm et al. (2020) |
| Culturomics      | +                            | +                | Clinically relevant taxa (-)           | X                   | High potential for further method development | Method development might have plateaued | Databases are still very limited | Lagger et al. (2018); Rahi and Valipour (2020) |
| Culture-independent | 16S rRNA/ITS amplicon sequencing | +                | Bacteria and archaea (++)              | ++                  | Improved resolution over short-read 16S rRNA amplicon sequencing | Method development might have plateaued | Many sources of bias | Johnson et al. (2019) |
| Full-length 16S rRNA amplicon sequencing | + | + | Bacteria and archaea (++) | ++ | Improved resolution over short-read 16S rRNA amplicon sequencing | Many sources of bias | Many sources of bias | Johnson et al. (2019) |
| Deep shotgun metagenomic sequencing | + | +++ | +++ | X X X +++ | - | Most comprehensive characterization of the whole microbiome | High fraction of sequencing effort in skin microbiome is spent on host genome | Quinones et al. (2017); Tatt et al. (2017); Liu et al. (2021) |
| Shallow shotgun metagenomic sequencing | + | ++ | +++ | X X ++ | - | Higher resolution compared to 16S rRNA amplicon sequencing with similar cost | High fraction of sequencing effort is spent on host genome | Hillmann et al. (2018, 2020); Cattano et al. (2020) |
| Metatranscriptomics | ++ | +++ | +++ | X X | NA Gene expression (+) | Metabolically active (X) | Simultaneous analysis of host and microbiome transcripts | Simultaneous analysis of host and microbiome transcripts (if not wanted) | Bailland et al. (2019); Shakya et al. (2019) |
| Metaproteomics   | ++ | +++ | Active microbiota (++) | X | Active metabolism (+) | Simplex analysis of host and microbiome transcript | Simultaneous analysis of host and microbiome transcript | Complements other analysis | Miles et al. (2019); Li and Figeys (2020) |
| Metabolomics     | ++ | +++ | Only in combination with other *omics | X | X | Metabolomics active (+) | Simultaneous analysis of host and microbiome transcript | Coverage of databases still needs improvement | Ribenstott et al. (2018); Bhawna et al. (2019) |
be predicted with amplicon sequencing based on the taxonomic identification of the organisms, but the accuracy of this approach is limited (Liu et al., 2021). The taxonomic resolution and other biases in these analyses are also affected by choice of primers, targeted variable regions, sequencing technology and downstream data analysis, and it is likely that no ‘best practices’ exist for 16S rRNA sequencing (Pollock et al., 2018). For example, the use of amplicon sequence variants has been recently studied as a more accurate alternative to OTUs in microbiota profiling (Callahan et al., 2017). However, all amplicon-based approaches are prone to similar biases (Nearing et al., 2021). Using either approach can result in the same biological conclusions with 16S rRNA amplicon sequencing (Moossavi et al., 2020), and the same issues apply to fungal amplicon sequencing (Nilsson et al., 2019). For example, specific primer sets for the 16S rRNA gene can sufficiently characterize skin bacteria (Castelino et al., 2017), but most universal primer pairs greatly overlook archaeal diversity (Bahram et al., 2019).

Furthermore, no fractional part of the 16S rRNA gene enables species-level resolution for bacterial identification (Johnson et al., 2019). Amplicon sequencing with short-read HTS platforms is thus not useful beyond genus-level resolution for archaea and bacteria. Thus, the choice of primers and the sequenced region of the target gene is always a compromise, which should be based on coverage of likely important taxa, and desired comparisons to previous studies and data.

Shotgun metagenomic sequencing means non-targeted sequencing of the total DNA in a sample (Quince et al., 2017). This is a powerful method that can potentially characterize all community members and their functional genes. Compared to amplicon sequencing, shotgun metagenomics can provide strain-level taxonomy of the organisms (Tett et al., 2017) and covers all components of the microbiome, including the virome and mobiome (mobile genetic elements; Carr et al., 2021). However, these benefits are offset by the increased sequencing effort and cost, the required biomass in the sample and more complex data analysis (Table 4; Liu et al., 2021). The use of shotgun metagenomics in skin microbiomes is further complicated by the high amount of host DNA in the samples. The host DNA sequences can be removed during data processing, but a large part of the sequencing effort is spent on sequencing of the human genome, which is usually an undesired outcome. However, both the cost of sequencing and template requirements have constantly been decreasing. In addition, the broader application of host DNA depletion methods could further increase the applicability of shotgun sequencing. The amount of bioinformatic methods available for shotgun metagenomic data is large enough to seem overwhelming for researchers entering this field. However, these can be roughly divided into assembly (or contig) based and read based approaches (Liu et al., 2021).

Briefly, in assembly-based methods, the sequencing reads are assembled into long contiguous pieces of DNA sequence, which are then matched against pre-existing databases to identify genes and organisms. The contigs can also be clustered to genomic bins based on, e.g. tetranucleotide frequency and uneven abundance of the contigs in different samples. The binning produces draft metagenome-assembled genomes (MAGs) and has proven to be a highly potent tool to examine the diversity of the skin microbiome (Li et al., 2021). Such analyses, however, require expensive deep sequencing of the samples and computational effort for assembly and binning, making them unsuited for researchers with limited budgets and resources, especially if a high number of samples are involved.

Read-based methods characterize the taxonomic and functional diversity of the community by aligning individual reads from HTS to reference databases. The matching is usually based on clade-specific marker genes, and in some methods, the taxonomic coverage has included all prokaryotic and eukaryotic microbes and viruses (Truong et al., 2015). While these tools generally have higher taxonomic coverage and lower bias than amplicon sequencing, their results are, however, dependent on the quality of the available marker gene databases (Lugli et al., 2019). Recently, shallow shotgun sequencing has shown to be a cost-effective alternative to 16S rRNA amplicon sequencing (Hillmann et al., 2018). It enables taxonomic profiling (Hillmann et al., 2020) but not contig or genome assembly (Cattonaro et al., 2020). Therefore, for genome assembly, which often requires immense sequencing effort, associated costs can be lowered by combining short-read sequencing with emerging long-read sequencing technologies (Sanders et al., 2019).

Other culture-independent approaches

In addition to amplicon and shotgun sequencing, several other omics methods have been used to profile skin microbiomes without laborious and time-consuming culturing steps. The three approaches introduced here, metatranscriptomics, metaproteomics and metabolomics, focus on analysing the function of the microbial community at the time of sampling (Fig. 2; Table 4; Aguiar-Pulido et al., 2016). This information is invaluable in understanding how the microbial ecosystem reacts to different stimuli and interactions within it and between the host. Similar to patterns detected with metagenomics, the other omics tools can also produce diagnostically useful signals, such
as skin microbiota-associated plasma metabolites as biomarkers for psoriasis (Chen et al., 2021).

In metatranscriptomics, mRNA present in the samples is extracted and reverse-transcribed to complementary DNA to facilitate its analysis and long-term storage (Shakya et al., 2019). In contrast to above-mentioned DNA-based analyses, metatranscriptomics directly profiles actively transcribed genes within the community, which are a proxy for its active metabolism at the moment of sampling. The current limitations of this method are the low coverage of existing reference databases, increased effort in sample preparation, high risk of contamination and mRNA degradation, and high cost (Sandhu et al., 2019). Despite these issues, metatranscriptomics has a high potential for elucidating the response of the skin microbiome to various conditions related to skin diseases. For instance, it has been used to show that specific pathways in Cutibacterium acnes (formerly Propionibacterium acnes) respond to vitamin B12 supplementation and are involved with the development of acne (Kang et al., 2015).

In metaproteomics, proteins extracted from a sample are most often separated with liquid chromatography and detected with mass spectrometry (Wilmes and Bond, 2006). This analysis produces spectral data which are referenced to pre-existing databases to identify peptides and proteins, their abundances, and the presence of any post-translational modifications, such as phosphorylation and acetylation. Metaproteomics is complementary to the other omics methods as it provides very accurate information on the function of the community at the time of sampling. However, it similarly suffers from laborious preprocessing steps, lacking reference databases, and challenges in data processing because of the high amount of data produced (Li and Figeys, 2020). It is also not as well suited for skin microbes since it requires a somewhat high amount of biomass, often obtained through enrichment steps (Petriz and Franco, 2017). However, recent developments have led to a more simplified laboratory and data production workflow, which might be more applicable for skin studies (Heyer et al., 2019).

In metabolomics, the cellular metabolic products in the skin or other tissue samples are extracted, characterized and quantified (Ribbenstedt et al., 2018). Most commonly, an extract of the metabolites is separated into components with liquid or gas chromatography (L/GC), and the compounds are characterized with mass or nuclear magnetic resonance spectrometry (MS/NMR; Ribbenstedt et al., 2018; Emwas et al., 2019). In non-targeted metabolomics, the whole range of non-identified compounds in the sample is correlated with an experimental condition. In the targeted approach, only specific metabolites matching a standard sample are detected. This approach is highly adept at detecting small compounds produced by the microbiota and is often combined with amplicon or shotgun metagenomic sequencing. Similarly to metaproteomics, the high cost, laborious sample preparation and sparse reference databases have however limited its applications in microbiomes (Sandhu et al., 2019). In skin microbial communities, metabolomics has been used to characterize biomarkers of healing wounds and their connections with specific bacteria in the associated microbial community (Ashrafi et al., 2020). Metabolic analysis of blood plasma integrated with metagenomic profiling of the skin microbiome has also detected microbiota-associated metabolites with potential as diagnostic markers for psoriasis (Chen et al., 2021).

Data analysis
A large variety of computational techniques and tools have been developed to process amplicon and shotgun sequencing and other types of omics data from microbiomes. Several choices need to be made in the bioinformatic and data science steps of a skin microbiome project (Fig. 2), and the number of available options is immense. Invaluable microbiome and metagenome analysis software and toolkits are available today, such as Anvi’o (Eren et al., 2021), bioBakery 3 (Beghini et al., 2021), QIIME2 (Bolyen et al., 2019), phyloseq (McMurdie and Holmes, 2013) and the R/Bioconductor ecosystem (Gentleman et al., 2004). They provide standardized solutions for most statistical and data analytical challenges related to the relevant omics data types while allowing considerable flexibility to design and implement custom workflows (Shetty and Lahti, 2019). Following the general developments in human microbiome studies, skin microbiome research increasingly employs multiple parallel omics assays, complementing metagenomic measurements with transcriptomics, metabolomics, single-cell sequencing and other omics technologies. Thus, appropriate statistical techniques (Sankaran and Holmes, 2019b) and ways to organize complex multi-assay data sets to facilitate smooth quantitative analyses are needed. Data science solutions, such as the TreeSummarizedExperiment data container (Huang et al., 2021), have been recently proposed as a dedicated approach to deal with such data aggregation tasks. While many tools can produce robust results, each method is prone to specific biases (Lugli et al., 2019; Moreno-Indias et al., 2021). Therefore, the evaluation of skin microbiome studies should begin with the description of participants and related metadata. This should continue to detailing of the data production, processing and analysis, through interactive and reproducible workflows and electronic notebook environments to ensure complete transparency, reproducibility and
While a thorough assessment of the methodology for microbiome data analysis is beyond the scope of this study, we can recommend reviews on the available tools and give recommendations for different purposes (Knight et al., 2018; Gardner et al., 2019; Shetty and Lahti, 2019; Liu et al., 2021). Challenges in the study of skin microbiomes, such as the large quantity and dimensionality of the data, and its compositionality (Gloor et al., 2017), are similar to those encountered in the study of other human microbiomes. Notably, compared to other body sites, such as sites along the gastrointestinal tract, the high variability of skin microbiome composition and the low biomass on skin adds further uncertainty in the analyses. Thus, probabilistic and latent variable techniques could further aid in quantifying associated uncertainties and improving the robustness of analyses when sample sizes or read counts are small, and uncertainty is high (Äijö et al., 2018; Metwally et al., 2018; Sankaran and Holmes, 2019a; McGhee et al., 2020; Marcos-Zambrano et al., 2021). Furthermore, the reproducibility crisis in science (Baker, 2016) also reaches the study of human microbiomes, especially with the recent surge in studies utilizing machine learning (Moreno-Indias et al., 2021). The widespread use of improper practices can be seen, for example, in gut microbiome studies utilizing machine learning (Quinn, 2021). These issues can likely also affect skin microbiome studies. Thus, more robust practices should be adopted in machine learning, such as using nested cross-validation and completely separating the training and test (or validation) data (Vabalas et al., 2019).

To facilitate progress in skin microbiome research through adoption of open science practices, we recommend that the data and analysis code be made findable, accessible, interoperable and reusable (FAIR; Moreno-Indias et al., 2021). For these purposes, minimum information about any sequence metadata standards has recently been developed (Bowers et al., 2017), and adopted by several databases, such as NCBI and EMBL. The use of MiXS and other suitable metadata standards is recommended because these standards cover the most critical parts of the data production and processing in microbiome studies and further the FAIR principles (Vangay et al., 2021).

**Perspectives**

The understanding of the structure and function of the skin microbiome has greatly increased during the last decade. This progress has been primarily fuelled by modern omics tools, especially amplicon sequencing and metagenomics. Here, we have identified several technological advancements and recent maturation of existing tools which could grant even further insights into the skin microbiomes and facilitate their future practical applications.

A number of future applications based on analysis of skin microbiomes can be envisioned in the field of forensics (Ahannach et al., 2021b). Such applications are possible, because humans leave traces of their skin microbial signature on touched items (Knights et al., 2011). Skin microbial communities are also stable over long periods on the same site on an individual (Oh et al., 2016). Thus, skin microbiomes might be proven useful in tracking contacts between people and objects to provide trace evidence for criminal cases and personal identification (Tozzo et al., 2020). Microbial source tracking has been developed and utilized primarily for tracking faecal contamination and pathogens in water (Boehm et al., 2013). Forensic application of skin microbiomes has recently been reported, utilizing both metagenomics sequencing (McGhee et al., 2020) and 16S rRNA amplicon sequencing data (Carter et al., 2020). High-resolution features of the skin microbiome appear to be most helpful in personal identification. Analysis of rare prokaryotic taxa on forehead skin with 16S rRNA amplicon sequencing correctly identified 78% of 89 participants when several samples per person were analysed (Watanabe et al., 2018). Nucleotide diversity of subsets of clade-specific markers from shotgun metagenomic sequencing was analysed to achieve, e.g. 96% identification accuracy on palm skin samples from 12 participants (Schmedes et al., 2017). Recently, amplicon sequencing of prokaryotic clustered regularly interspaced short palindromic repeats provided 95% accuracy in identifying 14 participants (Toyomane et al., 2021). Despite these promising results, there remain some practical and legal considerations that need to be addressed before the widespread adoption of microbiome-based methods in forensics (Clarke et al., 2017; García et al., 2020). For example, to the best of our knowledge, population-scale studies on the forensic utility of skin microbiome analysis have yet to be reported. Furthermore, addressing issues related to bias introduced in, e.g. sampling, sample handling and storage and standardization of analyses, highlighted in our review, would also likely increase the applicability of skin microbiomes in the forensic field.

While most of the current data in skin microbiomes are produced with potent HTS methods, such as Illumina’s MiSeq and Thermo Fisher’s Ion Torrent, these technologies have produced read lengths only of a few hundred base pairs. This can be problematic for amplicon and shotgun metagenomic sequencing because short amplicons on the 16S rRNA gene allow only genus-level taxonomic identification, and genome assemblies based on short reads can be highly fragmented. Long-read
 technologies, such as Sequel II from Pacific Biosciences, and MinION from Oxford Nanopore Technologies feature up to 50 kbp (PacBio) or 100 kbp (Nanopore) library insert sizes with average read lengths of over 10 kbp (Amarasinghe et al., 2020). Also, the previously high error rates of these methods are currently down to single digits (<1%) for PacBio and (<5%) Nanopore. This has made them highly useful in, e.g. hybrid genome assembly when combined with high-quality short-read data (Xie et al., 2020). The use of long-read platforms should be further explored for full-length 16S rRNA amplicon sequencing for strain-level characterization of communities (Johnson et al., 2021). In long-read sequencing, it should be noted that fragmentation of extracted DNA can present further issues (Maghini et al., 2021). Thus, the potential of high-molecular-weight DNA extraction (Maghini et al., 2021) and host DNA depletion (Marotz et al., 2018) could be investigated in the context of skin microbiomes to improve the template quality, especially for long-read shotgun metagenomics.

In read-based metagenomics, the originating species for functional genes is often uncertain or completely unknown. Furthermore, in assembly-based metagenomics, the contigs and MAGs are only average representations of the genomic content in the actual microbes in the samples. These issues could likely be addressed with single-cell metagenomics, where the genomic contents of individual microbial cells isolated from the sample are analysed separately (Xu and Zhao, 2018; Sharma and Thaisis, 2020). Some of the first applications of such single-amplified genomes (SAG) in microbiome research are highly promising. For example, the analysis of SAGs from uncultured strains enabled a detailed reconstruction of dietary fibre fermentation pathways in the mouse gut (Chijiwa et al., 2020). However, like other promising methods, several issues need to be addressed before the widespread adoption of single-cell metagenomics. For example, in skin microbiomes, the isolation of individual microbial cells from the samples using microfluidics (Tan and Toh, 2020) could aid with the issue of extensive host DNA contamination in metagenomic analyses.

The data analysis in host-associated microbiomes, such as the human skin microbiome, is currently dominated by traditional statistics, including distance-based methods (Ruukskan et al., 2021). However, these often do not account for many key features of microbiome profiling such as compositionality, heteroscedasticity, non-linear relationships, hierarchical and spatial structures, and functional redundancy. In addition, skin microbiomes display extensive spatial variation between individuals because of their close connection with the environment (Vandegrift et al., 2019). Spatial information alone could likely also serve as a proxy for variables with uneven spatial distributions, such as differences in surrounding vegetation and lifestyle-related factors. Thus, spatial modelling methods could likely improve their use in microbiomes in disease diagnosis and prediction.

Concluding remarks

The increased application of long-read sequencing and single-cell metagenomics is now shifting the research focus from mere correlations towards a more mechanistic understanding of the skin microbiomes. Understanding the dynamics of skin microbiomes at the mechanistic level is a prerequisite for future applications in forensics and medicine. However, the limited reproducibility has been slowing down the overall progress in skin microbiome studies. The reproducibility of research could be improved with standardization of the methods, combined with transparent reporting and open research practices (Moreno-Indias et al., 2021). More diverse data sets need to be collected to account for differences in host-associated and external factors affecting skin microbiomes, such as ethnicity, sex, age and the environment.

Author Contributions

M.O.R., D.V., R.P., A.R., R.A. and L.L. designed the work. M.O.R., D.V. and R.P. drafted the manuscript. M.O.R. drew the figures. A.R. and E.M. provided critical feedback and suggestions. R.A. and L.L. supervised the work. All authors edited and approved the final version.

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