Performing Skin Microbiome Research: A Method to the Madness

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Growing interest in microbial contributions to human health and disease has increasingly led investigators to examine the microbiome in both healthy skin and cutaneous disorders, including acne, psoriasis, and atopic dermatitis. The need for common language, effective study design, and validated methods is critical for high-quality standardized research. Features, unique to skin, pose particular challenges when conducting microbiome research. This review discusses microbiome research standards and highlights important factors to consider, including clinical study design, skin sampling, sample processing, DNA sequencing, control inclusion, and data analysis.

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INTRODUCTION

The relationship between host and cutaneous microbes has been of great clinical scientific interest, often studied with traditional cultivation methods and focused on a single/few bacteria (Evans et al., 1950; Kligman et al., 1976; Lai et al., 2010; Marples, 1965; Nizet et al., 2001). Reduced costs and increased access to high-throughput sequencing have enabled global examination of the skin microbiome, broadly defined as skin microbiota with their genomes and surrounding environmental conditions (Marchesi and Ravel, 2015).

Early skin microbiome studies described healthy human skin microbial communities as more diverse than those previously recognized through cultivation methods (Dekio et al., 2005; Gao et al., 2007; Grice et al., 2008) and unique to skin (Costello et al., 2009; Human Microbiome Project, 2012a, 2012b). Several reviews (Clavel et al., 2016; Goodrich et al., 2014; Huttenhower et al., 2014) have outlined important elements of high-quality microbiome studies. The unique aspects of skin, including low microbial biomass, high contamination risk (Salter et al., 2014), accessibility and diversity of cutaneous habitats, site-specific microbiota, and a distinct immune system (Naik et al., 2012; Watanabe et al., 2015), necessitate important considerations for conducting skin microbiome studies (Figure 1). Several reviews have summarized skin microbiome literature (Edmonds-Wilson et al., 2015; Jo et al., 2016b; Schommer and Gallo, 2013; Zeeuwen et al., 2013).

In emerging fields, studies to identify optimal methodologies are often performed, and several include elements related to the skin microbiome (Human Microbiome Project, 2012a, 2012b). Study design for skin microbiome research is multifaceted and integral to all downstream steps. Published studies examined skin sampling methods (Chng et al., 2016; Grice et al., 2008), sample storage (Lauber et al., 2010), controls and contamination sources (Salter et al., 2014), sequencing biases (Meisel et al., 2016), and possible quantitation (Gao et al., 2010). The current review integrates this combined expertise and focuses on the methodology and challenges of factors important for skin microbiome research to promote reliability and comparability (Figure 1). Of note, we primarily discuss 16S ribosomal RNA (rRNA) gene amplicon sequencing as the most widely used method.

POTENTIAL PITFALLS

Similar to other interdisciplinary fields, multiple factors are important in conducting or assessing a skin microbiome study.

- *Study design*: consistent metadata collection; considering potential confounding factors
- *Skin sample collection/storage*: standardized collection/handling of samples
- *Sample processing/sequencing*: DNA extraction; PCR conditions, primer selection
- *Process controls*: negative/blank controls; mock community comparison
- *Analysis methods*: pipeline description; sequencing data availability with associated metadata

STUDY DESIGN

Because the skin microbiome comprises different microbes including bacteria, fungi, and viruses, whether the scientific focus is on one particular kingdom or all microbes will

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Abbreviations: OTU, operational taxonomic unit; rRNA, ribosomal RNA; V, variable

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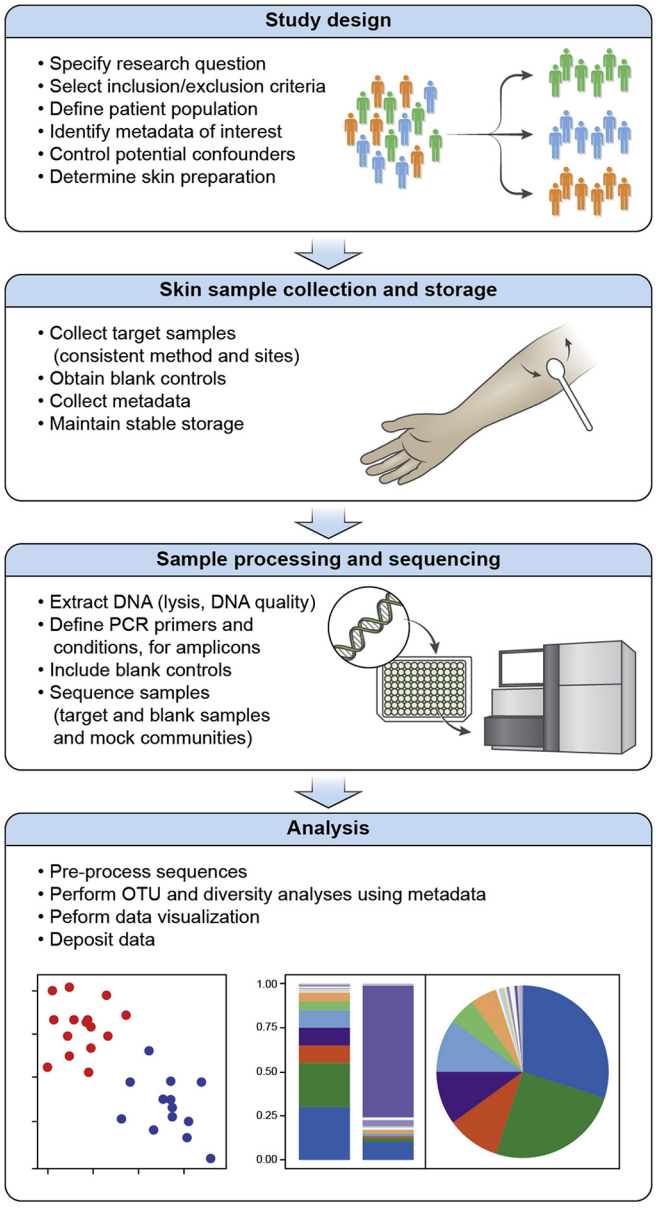

Figure 1. Steps for performing a skin microbiome study. The multiple elements of a skin microbiome study begin with study design, followed by skin sample collection and storage, sample processing and sequencing, and analysis. OTU, operational taxonomic unit.

influence study design, sequencing, costs, and analysis. Bacteria have been the main focus, but several have used sequencing methods to examine skin fungal (Findley et al., 2013; Jo et al., 2016a; Paulino et al., 2006; Zhang et al., 2011), viral (Foulongne et al., 2012; Hannigan et al., 2015; Oh et al., 2014, 2016; Wylie et al., 2014), and archaeal communities (Probst et al., 2013).

A "study population" may refer to individuals with/without a particular disease in a specific age range (Capone et al., 2011; Costello et al., 2013; Dominguez-Bello et al., 2010; Oh et al., 2012; Ying et al., 2015) or in a geographic region (Blaser et al., 2013; Clemente et al., 2015). Studies have demonstrated some interindividual differences in the skin microbiome even when matched for body site and sexual maturity, highlighting the need for the study design (e.g., sample size) to account for a certain degree of heterogeneity in the skin microbiome of a target study population; however, many features of the skin microbiome can be commonly observed (i.e., sebaceous sites hosting lipophilic bacteria). Skin bacterial communities in neonates, infants, and young children are notably distinct from those in sexually mature children and adults, particularly at certain skin sites (Capone et al., 2011; Costello et al., 2013; Dominguez-Bello et al., 2010; Jo et al., 2016a; Oh et al., 2012; Ying et al., 2015). The skin microbiomes in patients with different cutaneous and general medical conditions show distinctive patterns, but heterogeneity in the experimental study designs highlights the challenges in comparing results between studies and emphasizes a need for minimal standards.

Screening subjects involves collecting demographic data, obtaining detailed history on prior and/or current medical conditions and topical/systemic medications, performing clinical examinations, and considering diagnostic criteria. Explicit criteria for defining healthy individuals are important (Aagaard et al., 2013). Disease phenotyping (validated diagnostic criteria, severity scoring, and clinical photography) enables a more accurate comparison of subpopulations within a particular disorder.

A typical exclusion criterion for healthy individuals is prior systemic antibiotic usage, based on antibiotic use within the last 12 months (Grice et al., 2009), 6 months (Costello et al., 2009; Findley et al., 2013; Human Microbiome Project, 2012b), or 1 month (Gao et al., 2007). For individuals with skin disorders, prior usage of topical and systemic medications can affect the skin microbiome (Kong et al., 2012). This information can be used as exclusion criteria or as defining metadata. Other medications may influence the skin microbiome, and collecting a complete medication history is desirable.

Clinical metadata documentation is critical for downstream analyses and may help explain differences within/between studies. Commonly collected metadata include age, sex, antibiotic use, and sampling sites. Some factors such as pet ownership (Song et al., 2013), deodorant usage (Callewaert et al., 2013), physical activities (Meadow et al., 2013), season, time of day, country of birth, race/ethnicity, mode of delivery, and diet may influence the skin microbiome.

Calculating sample sizes for skin microbiome studies can be difficult without pre-existing data for estimating effect sizes. A few methods have been proposed to calculate sample sizes (Kelly et al., 2015; La Rosa et al., 2012), including a web-based tool called Evident (https://github.com/biocore/Evident). With growing numbers of skin microbiome studies, pre-existing data for estimating potential effect sizes are increasingly available for use in designing well-powered studies.

Skin preparation

Questions often arise regarding factors to control in skin microbiome studies. Standardizing controllable factors reduces confounders, maximizing the ability to determine the experimental variable responsible for any observed difference. Factors that can alter bacterial communities include hand-washing (Fierer et al., 2008) and application of non-

skin bacteria (Costello et al., 2009; Two et al., 2016). Because of potential fluctuations in skin microbial communities in the hours after bathing/showering, several studies have utilized a minimum number of hours after baths/showers before sampling; the minimum time since last washing has ranged from 12 hours (Human Microbiome Project, 2012a, 2012b) to 24 hours (Grice et al., 2009; Oh et al., 2012, 2016). Skin hygiene (e.g., soap and shampoo practices) can also alter the skin microbial communities in some circumstances (Perez Perez et al., 2016) but not in others (Two et al., 2016). Use of emollients can influence the skin microbiome (Seite et al., 2014); therefore, the restrictions on emollient use in studies have varied from 1 to 7 days of avoidance before sampling (Human Microbiome Project, 2012a, 2012b; Kong et al., 2012). The effects of topical medications, for example, steroids, on the skin microbiome can persist for up to 7 days (Kong et al., 2012).

SKIN SAMPLING METHODS

Multiple methodologies have been documented and validated for skin microbiome sampling, including swabs, biopsies, surface scrapes, cup scrubs, and tape strips (Chng et al., 2016; Costello et al., 2009; Findley et al., 2013; Gao et al., 2007; Grice et al., 2008; Nakatsuji et al., 2013; Oh et al., 2014; Zeeuwen et al., 2012). Each method varies in biomass yield, human DNA contribution, sampling depth, and discomfort level. The method should be based on scientific question, study design, study population, and sampling sites. The skin microbiome composition at the skin surface can differ from deeper layers of the skin (Grice et al., 2008; Nakatsuji et al., 2013; Zeeuwen et al., 2012) and between skin subcompartments and structures. Thus, consistency is key for skin microbiome sampling methods.

The most established collection method is premoistened swabbing (Aagaard et al., 2013; Human Microbiome Project, 2012a, 2012b; Paulino et al., 2006). Dry swabbing (Schowalter et al., 2010) has not been as widely utilized, due to reduced biomass collection. Tape stripping provides a reproducibly high amount of biomass (Chng et al., 2016) with stratum corneum depth analysis in a defined collection area (Zeeuwen et al., 2012). Tape stripping is not suitable for all body sites because of its dimensions and sample acquisition time. Scrapes increase biomass collected and could be useful for studies of low abundance microbes, for example, fungi (Findley et al., 2013; Grice et al., 2008). Cup scrubbing was pioneered for culture-based studies (Williamson and Kligman, 1965) and can also be used for microbiome studies (Chng et al., 2016). Skin punch biopsies enable the analysis of microbial DNA potentially deeper in skin. In addition to tissue homogenization for total DNA collection, a portion of skin biopsies can be processed for other analyses, possibly laser capture microscopy (Grice et al., 2008; Nakatsuji et al., 2013). However, biopsies are invasive with reduced ability to sample multiple sites in patients.

Most major bacterial taxa are similarly identified in swabs, scrapes, and biopsies, but a tree-based analysis could segregate sampling methods (Grice et al., 2008). Comparing swabs, tape strips, and cup scrubs for 16S rRNA gene or shotgun metagenomic sequencing provided high concordance when analyzing relative abundance (Chng et al., 2016). While highlighting that multiple methods can be used, it is important to maintain a consistent standardized approach for sample collection throughout a study.

Other aspects of sample collection have been emphasized in prior studies, that is, location, sampling frequency, and use of controls. Skin microbial communities exhibit striking site-specific differences and notable topographical diversity over the human skin surface, even within the same individual. Yet, regions of skin with common physiological characteristics—sebaceous, moist, dry—share some similarities in the composition of skin microbial communities (Costello et al., 2009; Grice et al., 2009; Oh et al., 2012, 2014). Consistent sampling of the same anatomic area in the entire study cohort reduces confounders and maximizes the ability to identify microbiome differences (Costello et al., 2009; Grice et al., 2009). Sampling body sites with bilateral symmetry has also been used to confirm that consistency is observed with low intraindividual variability (Chng et al., 2016; Grice et al., 2009). Frequency of sampling has varied in different studies from a single timepoint to repeated sampling. Longitudinal sampling of skin has demonstrated that skin microbiomes are highly individualized (Flores et al., 2014; Oh et al., 2016), which suggests that repeated sampling can provide an internal control and help to increase the statistical power for analyzing changes in a chronic skin disorder (Kong et al., 2012).

Sample storage

For sample handling, most studies have used the standard method of immediate freezing after sample collection, followed by storage at −80 °C (Blaser et al., 2013; Costello et al., 2009; Grice et al., 2009). No major differences in the bacterial communities were found in skin and fecal samples collected from two individuals and stored at different temperatures for 2 weeks (Lauber et al., 2010). However, storage at −80 °C is generally advised (Goodrich et al., 2014). Studies have demonstrated that freeze-thaw cycles can alter the microbial composition observed in samples and should be avoided (Cuthbertson et al., 2015; Sergeant et al., 2012).

SAMPLE PROCESSING

DNA extraction

During early steps of sample preparation, bias can easily be introduced (Brooks et al., 2015; Yuan et al., 2012), especially during the lysis of microbial cells (Yuan et al., 2012). Mechanical lysis (with or without enzymatic treatment) provides more comprehensive profiles of Gram-positive bacterial populations and fungi than enzymatic treatment alone, and is thus strongly advisable (Albertsen et al., 2015; Findley et al., 2013; Santiago et al., 2014; Sergeant et al., 2012; Walker et al., 2015; Yuan et al., 2012). After lysis, DNA can be purified following different approaches, for example, by alcohol precipitation or via binding to affinity columns. Downstream applications will dictate genomic DNA requirements: higher standards in terms of fragment size, amount, and purity of target DNA are often required for some sequencing methods. Therefore, consistent documentation of the quality and quantity of DNA obtained (e.g., fluorometry, UV spectroscopy) is important (Olson and Morrow, 2012) and performing

gel electrophoresis on high yield samples will help to characterize fragment size distribution.

Library construction: amplicons

PCR amplification introduces biases in datasets. Use of PCR replicates, controls, appropriate PCR primers, and PCR cycle conditions helps control library quality. PCR replicates (at least duplicates; if possible triplicates) that are pooled in downstream steps (e.g., during library purification) can be prepared to limit effects of early amplification bias (Acinas et al., 2005).

Primer choice significantly influences microbial profiles as PCR efficiency can vary between organisms (Hiergeist et al., 2016; Meisel et al., 2016; Walker et al., 2015). The most commonly used gene in amplicon metagenomics studies is the 16S rRNA gene. Several sets of 16S rRNA gene-specific primers have been designed, targeting different variable (V) regions and amplicon sizes (Klindworth et al., 2013). Multiple studies have evaluated primer combinations and compared the utility with different sample types. Primer region selection depends on the scientific question and potential bacterial taxa of interest (Human Microbiome Project, 2012a). *Staphylococcus* and *Streptococcus spp.* are major skin bacteria best distinguished using V1 and V2 regions (Conlan et al., 2012). A recent study showed that products spanning V1−V3 were more accurate in classifying common skin bacteria and were more similar to results obtained using shotgun metagenomic sequencing of a mock community, as compared with V4 (Meisel et al., 2016). The currently most used region for analysis of skin bacterial communities is V1−V3. Consistent use of a single target region facilitates a comparison between studies yet may limit differentiation of bacteria optimally sequenced with other primer regions. Fungal sequencing studies have targeted a few different regions associated with the 5.8S, 18S, 28S, and internal transcribed spacer 1/2 ribosomal genes (Findley et al., 2013; Paulino et al., 2006; Wang et al., 2015; Zhang et al., 2011).

A key PCR parameter is the number of cycles used for amplification. An increase in the number of amplification cycles can result in lower diversity, and can skew bacterial profiles toward detection of low abundant taxa (Acinas et al., 2005; Bonnet et al., 2002). Hence, it is advisable that PCR cycles are kept to the lowest number that still delivers reproducible DNA amounts across a wide range of samples while also avoiding overamplification and ensuring that negative controls do not yield a product. Several groups have used approximately 30 cycles of PCR (Dekio et al., 2005; Gao et al., 2007; Grice et al., 2009; Human Microbiome Project, 2012a, 2012b; Smeekens et al., 2014). The quality of the amplicon libraries produced should be documented, for example, by controlling for the presence of unspecific bands using high-sensitivity electrophoresis.

SEQUENCING

Different sequencing platforms are available with various chemistries and technologies, and selection of the platform will be dependent on sequencer availability, amplicon size, sequencing depth, sequencing accuracy, and/or budget. With increased sequencing capacity, it has become feasible to routinely carry out shotgun metagenomic sequencing from human skin samples (Chng et al., 2016; Kang et al., 2015; Oh et al., 2014, 2016). Some advantages of this strategy include reduced amplification bias, generation of multikingdom genetic information, strain identification, and detailed genomic coverage for prediction of functional capacity (Chng et al., 2016; Human Microbiome Project, 2012b; Oh et al., 2014, 2016). Challenges with whole metagenomic sequencing are associated with data analysis complexity and low biomass from human skin samples, typically in the pg to ng range, which can make sequencing library construction difficult and increases sensitivity to contamination with mammalian DNA and microorganisms from the environment and laboratory reagents.

USE OF BLANK AND CONTROL SAMPLES

For the low biomass of skin samples, blank and control samples must be collected and run in parallel to target samples (Costello et al., 2009; Grice et al., 2009; Paulino et al., 2006). Blank samples are controls intended to contain no biological starting material of interest (e.g., only collection/storage buffers), including mock samples collected during patient sampling (e.g., premoistened swab fanned in the air) and DNA extraction processes, and PCR/sequencing blanks (containing only purified PCR-grade water as template). Blanks are processed with target samples to identify extraction contaminants and amplification artifacts, cross-contamination during library preparation, and contaminants entering during sequencing.

Mock communities (artificial mixtures of known target microorganisms) and reference samples of known composition are critical for benchmarking sample processing and sequence analysis (D'Amore et al., 2016; Jumpstart Consortium Human Microbiome Project Data Generation Working, 2012; Yuan et al., 2012). A mock community used with each sequencing run or across sequencing centers can promote standardization (Sinha et al., 2015) and should include multiple taxa important to the ecosystem of interest. A widely available synthetic DNA mock community (BEI Resources HM-276D) has been developed (Jumpstart Consortium Human Microbiome Project Data Generation Working, 2012); this includes bacterial species important to skin (*Propionibacterium acnes, Staphylococcus aureus,* and *Staphylococcus epidermidis*).

Additionally, when studying a patient cohort, it would be ideal to leverage data generated from healthy volunteers in previous small and larger studies. Although in principle this increases the power of the study, collection and analysis of data from some healthy volunteers alongside the patient population of interest would control for factors specific to the study.

ANALYSIS METHODS

The quality of analysis is dependent on consistent and thorough documentation of clinical metadata, sample collection/storage, DNA processing of target samples, negative controls, mock community positive controls, and sequencing methods (Goodrich et al., 2014). The metadata allow researchers to delineate potential causes/associations with sequencing results. Analysis pipelines, for example, QIIME (Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2010),

mothur (Schloss et al., 2009), and IMNGS (Integrated Microbial Next Generation Sequencing) platform (Lagkouvardos et al., 2016), have tutorials and best practices with parameters that should be evaluated for appropriate use with a particular dataset. There are many approaches to analyzing 16S rRNA gene sequencing data and the following overview briefly highlights most commonly used methods.

The 16S rRNA gene sequence analysis pipeline comprises three main components: preprocessing sequences, constructing OTU (operational taxonomic units, similar to bacterial taxa or species) tables, and annotating OTU tables. Sequence preprocessing is used to remove low-quality sequences before OTU table construction. Chimeric sequences generated during the PCR amplification process also need to be identified and removed from the dataset (Haas et al., 2011). The appropriate analysis methods and parameters are dependent on the sequencing method and amplicon region (D'Amore et al., 2016).

After preprocessing, the OTU table is constructed by clustering (grouping) similar sequences based on a defined similarity threshold. There are several approaches to clustering (Chen et al., 2013). The reference clusters used and the similarity threshold significantly affect the clustering results (He et al., 2015; Kopylova et al., 2016; Schloss, 2016). Normalization or rarefaction is used to address issues due to sequencing depth differences (McMurdie and Holmes, 2014).

The resulting OTU table is annotated based on representative OTU taxonomic and phylogenetic relatedness, using specific databases (Conlan et al., 2012; Schloss, 2010). A number of different methods are used to classify 16S rRNA gene sequences, for example, reference-based clustering, sequence similarity (e.g., BLAST or Basic Local Alignment Search Tool), K-mer-based methods (e.g., RDP or Ribosomal Database Project), and phylogenetic placement (Nguyen et al., 2016). Phylogenetic trees are commonly used in diversity metrics (e.g., UniFrac) or for data visualization.

Analysis of shotgun metagenomic data is challenging due to the orders of magnitude larger amounts of data generated and requires filtering high percentages of human sequences (Chng et al., 2016; Oh et al., 2014, 2016). Limitations of microbial sequencing analyses relate to the need for more reference genomes and for fundamental research on the function of genes identified in metagenomic sequencing. Analytical methods will continue to evolve to utilize both reference-based and reference-free mapping strategies (Human Microbiome Project, 2012b; Ma et al., 2014; Oh et al., 2014; Wylie et al., 2014). For microbiome and other genomic data, depositing data with relevant metadata in public repositories is a standard requirement for many funding agencies and allows acceleration of research through data sharing, reanalysis, validation, and compilation.

GUIDELINES AND FUTURE NEEDS

Maintaining a standardized format for reporting sample handling and processing with a common scientific language (Marchesi and Ravel, 2015) is important to promote reproducibility and advance science (Ravel and Wommack, 2014). Minimal standards/guidelines are often developed to further these goals, for example, MIQE for quantitative real-time PCR (Bustin et al., 2009), MIAME (minimum information about a microarray experiment) for microarray studies (Knudsen et al., 2005), CONSORT for clinical trials (Begg et al., 1996), and MIMARKS (minimum information about a marker gene sequence) and MIxS (minimum information about any (x) sequence) for genomics (Yilmaz et al., 2011). Research utilizing large DNA sequence datasets requires significant data analysis with a unique set of criteria for reproducibility (Peng, 2011). Depositing primary data into public databases enables independent cross-evaluation and comparison with previous studies, but requires access to raw data, metadata, software, and code used to process the data (Sandve et al., 2013; Stodden et al., 2014). The Genomic Standards Consortium has promoted minimum information standards and provides checklists for a broad range of genomic studies (Yilmaz et al., 2011). Adapting existing guidelines to skin microbiome studies, key areas that are important to clearly describe in manuscripts and attach (as metadata) to sequencing data are as follows:

Study design: Include clinical protocol information, study population, inclusion/exclusion criteria, medical and medication history, clinical phenotyping/validated tools for diagnosis and/or severity assessment, documented clinical metadata, and skin preparation regimen.

Sample collection/storage: Include description of sampling methods, skin sites, sample storage, and controls.

Sample processing/sequencing: Include detailed lysis methods, DNA purification, PCR primers, PCR conditions, processing controls, and sequencing.

Process controls: Include negative controls during sample collection, processing, and sequencing and a mock community.

Analysis pipeline: Include software (version), any specific commands, parameter settings, statistical tests, scripts for new pipelines (if applicable), and reference databases (version, access date, and modifications).

Data deposition: Genomic data sharing is often required with publication in many journals. Note that submission of raw sequences to a publicly available database should be accompanied by specific relevant metadata, for example, collection date, skin site/location, clinical features (Yilmaz et al., 2011).

In addition, several areas for growth could facilitate advancement toward mechanistic studies that better elucidate host-microbial interactions. Skin microbiome studies are an important window into our microbial communities and enable formulation of hypotheses, but cause-or-effect relationships can be difficult to untangle. To test functional hypotheses, conducting studies that progress beyond DNA sequence data to include clinical isolates is key. Genomic and biological differences are well recognized at the microbial species and strain level, for example, *S. aureus* strains

with or without methicillin resistance (Greenblum et al., 2015; Oh et al., 2014). Thus, strains of a particular species available from biorepositories may or may not function in the same manner as the strains directly obtained in parallel from subjects studied as part of a microbiome analysis. Cultivating and curating skin-associated strains from human studies and making these available would improve sequencing analyses and provide live and highly relevant microbes for biological experiments. To isolate new reference strains, it is important to optimize methods for capturing a diverse set of skin microbes that reflects the complexity of the microbial communities as has been done for other body sites (Browne et al., 2016). Single-cell microbial sequencing could be a future option (Lasken and McLean, 2014), which would still benefit from reference genomes that take advantage of the relative ease of culturing most skin-associated bacterial strains.

Current methods to study the skin microbiome are based on relative abundance of microbes. Determination of bioburden and quantification of skin microbes is relevant to understanding the bioburden of microbes the skin typically harbors and whether it increases in certain diseases. One could perform quantitative PCR of the 16S rRNA gene to determine bacterial recovery in a given sample. A major issue is normalization; does one normalize to total DNA, which would include both human and microbial and could alter with disease state? Or does one normalize to surface area sampled, which might be affected by pressure applied and thus layers of skin released? In parallel with sequencing, developing microscopy methods to improve visualization of skin bacterial communities would greatly advance our ability to understand the structure and potential interactions within microbial communities.

Although skin microbiome studies may identify differences between affected and unaffected subjects, correlation-versus-causation questions remain. Longitudinal data across multiple timepoints can provide insights into the natural history of diseases and dynamics of ecological succession of the skin microbial communities. Another method to gather further evidence supporting causation is to identify colonization with a specific microbe or community of microbes before disease manifestation. Given the interest in microbial education of the immune system, birth cohort study designs provide an opportunity to obtain multiple skin samples before disease development, similar to published gut studies (Bokulich et al., 2016; Vatanen et al., 2016; Yassour et al., 2016). Meta-transcriptomics would also offer valuable information regarding the expression of microbial genes during the natural course of disease.

CONCLUSION

Studies of skin microbiome research have the potential to improve our understanding of host-microbial interactions. A byproduct of the expansion in the number of published skin microbiome studies is the need to understand how studies interrelate. Several scientific communities have developed minimal standards to improve the overall quality of different fields of research (Yilmaz et al., 2011). Minimal standards will contribute to the development of robust studies in skin microbiome research.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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