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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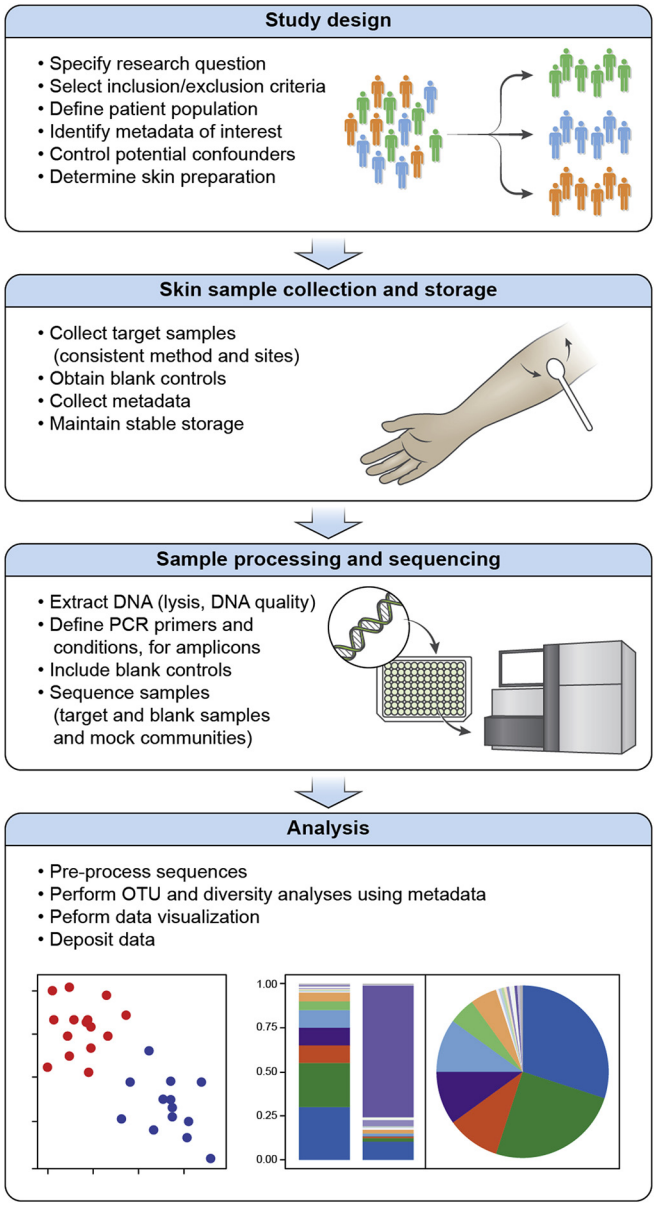

Figure 1. Steps for performing a skin microbiome study. The multiple elements of a skin microbiome study begin with study design, followed by skin sample collection and storage, sample processing and sequencing, and analysis. OTU, operational taxonomic unit.

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-