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Supplementary information

The human skin microbiome: from metagenomes to therapeutics

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Supplementary BOX 1| Technologies and challenges in studying the skin microbiome.

Long-standing and emerging technologies to study microbial diversity include (further reviewed in [1,2])

- Microbial culturomics: High throughput cultivation of (primarily) bacteria to obtain isolates that enable the generation of high-quality reference genomes, characterization of strain diversity, identification of patient-specific strains, experimentation with strain-specific phenotypes (e.g., [3]).
- Amplicon sequencing: Sequencing of a single marker gene from bacteria (e.g., 16S ribosomal RNA gene) and fungi (18S rRNA or internal transcribed spacer (ITS) region allows relatively inexpensive identification of the genera present in a sample. It can be a fragment of the marker gene (e.g., V1-V3, V4 of the 16S rRNA gene) to suit short-read sequencing technologies, or increasingly full-length is used with long-read technologies, which improves classification accuracy.
- Metagenomic whole genome sequencing (mWGS): Sequencing of the full complement of DNA genomes in a sample, which allows more precise reconstruction of microbial species present in a sample and their gene repertoire. Useful for studying microbial diversity, antibiotic resistance genes, metabolic potentials, and inferring growth rate of different microbes in the sample[4,5]. In addition, *de novo* assembly of long- or short-read metagenomic data, which does not rely on reference databases, allows reconstruction of bacterial, fungal, viral, or eukaryotic genomes that are not be present in current reference databases due to difficulty in their isolation. These metagenome-assembled genomes (MAGs) are now frequently added to databases to further describe microbial diversity[6].
- Propidium monoazide (PMA)-seq: Stains DNA from live vs. dead cells, followed by amplicon sequencing allows determination of bacteria viability[7,8].
- (Meta)transcriptomics: Bulk RNA-sequencing of host cells allows for identification of key genes and pathways influenced by skin microbes[9]; metatranscriptomics attempts to identify the bulk microbial transcriptional activity (though challenging in practice, for skin microbiota given low biomass[10]).
- Single-cell sequencing: High-throughput and multiplexed approach to characterize transcriptional heterogeneity and cell states of individual cells of microbial populations (e.g. BacDrop[11], M3-seq[12], ProBac-seq[13], microSPLiT[14]) could be applied to cultured skin microbes.
- Host–microbiome spatial transcriptomics: Combining resolution of single cell transcriptomes with the spatial organization of tissue imaging, this approach has promise to decipher the host-microbiome interactions at the transcriptional level, with a spatial context (predominantly applied to the gut[15,16])
- Metabolomics: Profiling of metabolites inside and outside microbes through versions of mass spectrometry or NMR by measuring (reviewed in[17]) can be applied to monocultures or microbial communities[18] and the skin microbiome in disease[19].

- Metaproteomics: Profiling of proteins in a microbial community to reveal the function of the microbiome.

Integration of multiple 'omics technologies will be instrumental to a higher resolution map of host-microbe interactions and community dynamics. Computational innovations, such as using machine learning and artificial intelligence are also needed. While numerous technologies have been developed, many have been difficult to apply to skin microbiota given sparse colonization resulting in low RNA, metabolite, or protein yields.

Supplementary BOX 2 | *In situ* microbiome engineering.

The studies below target the gut microbiome, but conceptually, are inspirations for skin.

Conjugation-mediated engineering allows plasmid exchange through cell-cell contact, offering a broader host range and capacity for complex genetic transfer compared to phages. In the XPORT system, miniaturized integrative and conjugative elements (ICEs) from *Bacillus subtilis* were developed to enable gene transfer across 35 diverse Gram-positive strains[20] and delivered multi-gene pathways into synthetic soil communities. The MAGIC platform used the RP4 conjugation system[21] for similar *in situ* genetic modification of both Gram-positive and Gram-negative microbiomes.

Phage-mediated engineering uses natural or engineered phages to target specific bacteria. While natural phages can have exquisite host specificity, their use has had limited success beyond large screens for identifying therapeutic phages. Phage engineering and recoding has begun to increase host range by changing tail fiber diversity or creating libraries of tail fiber variants to select for a desired host range. Exciting advances include precision elimination[22] and base editing of *E. coli in situ*[23] in the gut microbiome using CRISPR-Cas9 and base editor payloads by engineered phages. Both studies targeted antibiotic resistance genes in *E. coli* and achieved significant bacterial reduction (4 orders of magnitude), a deeply penetrant (>90%) and durable editing (~6 weeks) with minimal off-target effects. Conceptually, these approaches could extend to skin microbiota, where diverse *Staphylococcus* phages have been identified.

Supplementary BOX 3| Advances and limitations of common skin microbiome models. Given the ethical concerns, safety requirements, and potential adverse events, testing microbiome therapeutics *in vivo* presents numerous challenges.

In vitro models. Tissue culture models featuring keratinocytes, fibroblasts, sebocytes, immune cells, and other cells isolated from skin can be colonized by microbes or exposed to microbial supernatants

to understand that cell's response. However, these models lack the spatial organization inherent to skin microbiome interactions. These cells (or pluripotent stem cells differentiated into different cell types) can also be used to construct 3D models that may better recapitulate skin physiology[24] and are arguably more valuable for microbiome-interaction studies. While the most common variations of these 3D models, often referred to as reconstructed human epidermis (RHE), feature keratinocyte-only epidermis or keratinocyte and fibroblast epidermal and dermal layers), ongoing innovations in bioprinting seek to add immune cells, stem cells, and various skin appendages like hair follicles, to better model *in vivo* interactions, though the complexity of stably maintaining these diverse cell types remains a challenge.

Ex vivo human skin models are derived from human surgical tissue and preserve the full architecture of skin, including immune cells and appendages[25–27]. These models maintain tissue integrity for 7–14 days, allowing localized immune studies. However, donor variability and limited sample availability may affect reproducibility and throughput.

In vivo models. Mice and pigs are the primary *in vivo* models. Pigs share anatomical and physiological similarities with human skin, with microbiomes dominated by Firmicutes[28]. However, their high cost, low throughput, and care requirements are disadvantages. Mice, valued for genetic diversity and disease models, are widely used in dermatological research. Despite similarities in microbiome phyla, differences in skin structure, such as the lack of sweat glands, challenge the colonization of human-derived microbes. In addition, differences in mice between vendors and facilities[29] can impact reproducibility.

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