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Challenges Developing a Human Model System for Skin Microbiome Research

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TO THE EDITOR

The human skin is colonized by a diversity of microorganisms. The human skin microbiome has come into great interest for its role in skin health and disease, and as a potential target for therapeutic and cosmetic applications (Byrd et al. 2018). A major limitation in the laboratory investigation of microbial and host-microbe interactions on human skin is the lack of a model that can reliably reproduce the complexity of the host. Attempts to model human microbial communities in rodents are impeded by competition from native flora, and even germ-free models suffer due to the substantial differences between murine and human integumentary systems. Reconstructed Human Epidermis (RHE), epidermis generated from human stem cells and cultured on an air-liquid-interface, lack the histological, physiological, and immunological complexity of human skin. By contrast, skin explants from living human donors recapitulate with greater fidelity the skin's physiology, but may be subject to greater inter-donor variability. We performed a pilot of microbiome and skin microbial colonization experiments with both RHE and explants, and present our methods comparison here for the benefit of the community.

We sought to develop a standardized protocol in which defined microbial communities could be colonized onto a human skin model, intervened upon as required, and subsequently

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AUTHOR CONTRIBUTIONS

Conceptualization: JO, PK, DC, EF; Data curation: PL, JO, DC; Formal Analysis: PL, DC. Funding Acquisition: JO; Investigation: PL, DC, EF; Methodology PL, EF, DC; Project Administration: JO; Resources: EF, JO, PL; Software: PL, JO, DC; Validation: PL, EF, DC; Visualization: PL, DC; Writing Original Draft Preparation: PL; Writing Review and Editing JO, PL.

DATA AVAILABILITY

Datasets related to this article can be requested from the authors upon request.

CONFLICTS OF INTEREST

The authors state no financial conflict of interest. Epiderm and Labskin models were purchased under an academic discount from Mattek and Innoven, respectively. Genoskin provided the NativeSkin samples used in the study at no cost, in exchange for permission to use results.

analyzed. We tested three commercially available human skin models, all three cultured in an air-liquid interface. Epiderm (Mattek, Kubilus et al. 2004, Netzlaff et al. 2005) is an RHE differentiated from human foreskin-derived epidermal keratinocytes. In addition to a layer of epidermis, Labskin (Innoven, Holland et al. 2008, Harvey et al. 2016) adds a thick dermal matrix constructed from fibroblasts, and is specifically advertised as a model for microbiome colonization. Finally, NativeSkin (Genoskin, Wever et al. 2015, Abadie et al. 2018) are skin biopsies from living human donors, which contain the full architecture of the human skin, including epidermis, dermis, skin appendages, and native immune cells (Figure 1a).

RHEs are differentiated in a sterile laboratory environment and can be treated as a 'germ-free' model, accordingly. By contrast, explants from living donors, even when treated with antiseptics or antibiotics prior to surgery, may have a microbial background. To investigate this, we performed metagenomic whole genome shotgun (mWGS) and 16S rRNA amplicon gene sequencing on explants from four different donors (Figure 1, S1, Table ST1). We tested different microbiome collection methods (swabs, tissue dissociation, or whole-tissue homogenization) for facile investigation, particularly for shotgun metagenomics, whose sequencing depth can suffer significantly from human DNA admixture (Figure 1b–d). Dissociating and filtering samples yielded the highest number of unique species identified. However, whole-skin preparations, while comprehensive, result in exorbitantly high DNA admixture that limits recovery of microbial sequence reads. While facile, swab-based methods were more dissimilar, potentially because swabbing has limited recovery of adherent microbes or those in secondary structures (e.g., biofilms) or appendages. Generally, we observed high relative abundances of Actinobacteria (*Cutibacterium, Corynebacterium*) and Firmicutes (*Staphylococcus*), common dominant skin microbes (Oh et al. 2014). As expected, donor-donor variability exceeded variation observed within a donor (Figure 1e), though for all donors, interestingly, microbiome composition remained largely stable over the explant life (Figure 1f–g). This, taken together with an inability to recover live microbes via cultivation at either timepoint (in contrast to RHEs, which were readily cultivatable, Table ST2) suggested that the pre-treatments and/or subsequent tissue processing may have killed surface microbes, necessitating DNA-based methods to detect the variable donor background.

To compare how each system may be used for experimental skin microbiota modeling, we applied a mock bacterial skin community comprised of four common skin commensals. After establishing each of the models as recommended by the manufacturer, we applied microbes and collected samples after 5–7 days to allow the community to equilibrate (Figure 2a). By CFU analysis, qPCR, and 16S rRNA sequencing, we detected all predicted organisms on all models, with terminal composition varying by model and in the case of explants, per donor (Figure 2b–d). Labskin yielded the most consistent results (Figure 2c, S2). We noted an expansion of staphylococci in all models, and an expansion of the relative abundance of *Cutibacterium* only in the explant model. A potential explanation is that *Cutibacterium acnes* favors hypoxic, lipid-rich environments (Dréno et al. 2018), which are likely comparatively reduced or absent in RHE models. It is also possible that host-derived *Cutibacterium* could be expanding, although unlikely given the stability of no-microbe controls.

Finally, we compared 16S rRNA sequencing with qPCR, mWGS, and colony quantitation (Table ST2) for assessing model utility. We observed consistent disparities between the microbial presence implied by nucleic acid analyses and the minimal or absent culturable organisms recovered. To explain this disparity, we applied a tool previously developed by our group to infer microbial growth rate from mWGS sequence data (Emiola et al. 2018). For the samples and organisms with adequate sequence depth to apply GRiD, growth rate indices indicative of active growth suggested a trend towards congruency with increased relative abundance (Figure 2b) and to a lesser extent, the recovery of culturable organisms; however, this analysis was limited due to the low microbial sequencing depth recoverable from explants.

In our opinion, both RHE and explant models have advantages and disadvantages for skin microbiology assays. Our results suggest RHE models may yield more reproducible results, but may be less conducive to the study of aerotolerant anaerobes like *C. acnes* than explant models. Explants more faithfully recapitulate the human epidermis, which may facilitate the study of a wider range of microbes and microbial communities and interactions with the skin's innate immunity. However, residual antimicrobials from topical antisepsis and systemic presurgical prophylaxis may render the native microbiome of explant donors inert and interfere with experimentally applied organisms. Explants from non-prophylaxed donors that have been adequately cleaned prior to use may circumvent this issue. In addition, none of these models will be able to recapitulate human diversity that can affect microbiome characteristics, e.g., ethnicity, gender, age, genetic diversity; *ex vivo* models are skewed towards demographics that have higher elective cosmetic surgeries, and RHE are derived in batches from different foreskin donors. Finally, when applying high concentrations of bacteria to a small model, nucleic acid amplification assays are particularly prone to over-representing non-culturable and potentially dead organisms. Culture-based assays or high-depth mWGS allow greater confidence in the results of *ex vivo* skin community models.

Additional limitations common between these models include their short lifespan, which may limit studies with slower-growing microbes, biofilms, or those examining microbiome stability, lack of secondary structures (RHEs), and the relatively high cost per unit. Despite the above caveats, we believe that all of these models can be useful for studying short-term microbiome and host-microbiome interactions, and for safety testing and pathogen or probiotic intervention studies. In addition, we expect that they can be used to investigate important knowledge gaps in microbiome dysbiosis. For example, mock communities could be colonized onto skin models and intervened upon to cause or resolve dysbiosis. It is also possible that patient samples could, to a degree, be transferred to a model, but we expect there to be major technical considerations requiring optimization, including accurate representation of microbial load, diversity, live-dead, and presence in secondary structure. Furthermore, although not explored in this paper, fibroblast (Labskin) and resident leukocyte immune activation (NativeSkin) could be measured in response to model dysbioses. We hope this letter assists other researchers in their implementation of laboratory skin microbiome experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

RHE	Reconstructed Human Epidermis
mWGS	metagenomic whole genome shotgun sequencing

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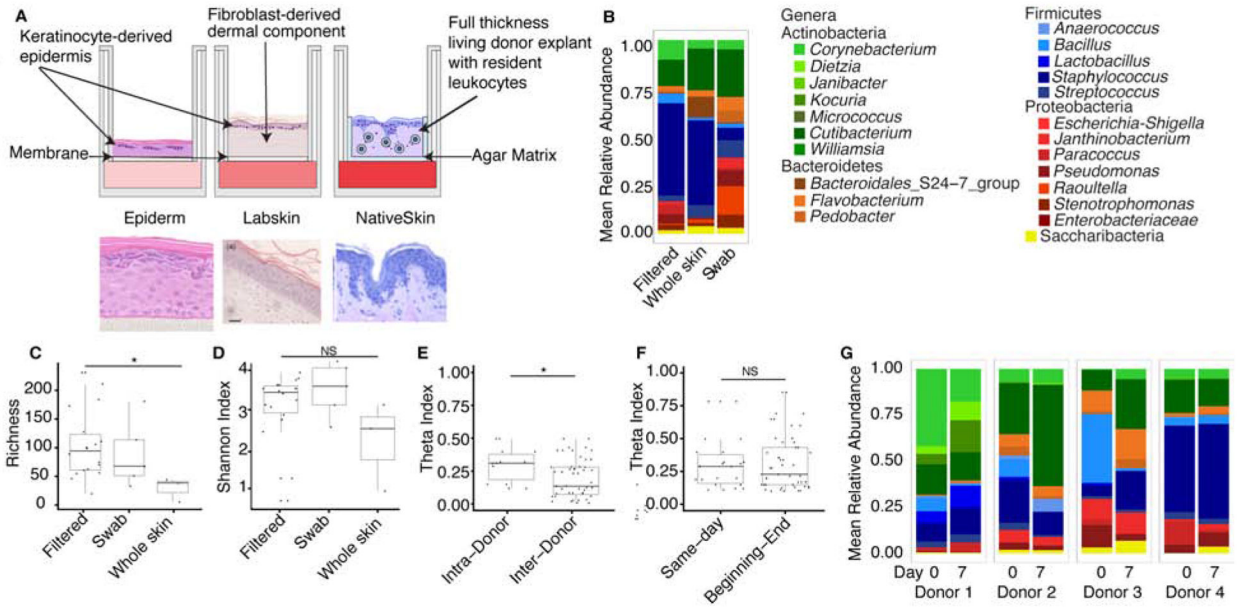

Figure 1:

Technical considerations for human skin microbiome modeling. a) Comparison of skin models used; Epiderm, Labskin, and NativeSkin, respectively, highlighting major differences with histologic representations obtained from manufacturer websites: Epiderm (Mattek website, https://www.mattek.com/wp-content/uploads/Histology-1-1.png, retrieved 2020), Labskin (Innovenn, image from Holland et al. 2008), NativeSkin (Genoskin website, https://www.genoskin.com/wp-content/uploads/2019/05/HE-1024×241.png, retrieved 2020). b) Comparison of microbiome collection methods. Barplots show mean relative abundance of a donor's microbiome obtained via tissue dissociation and filtration, whole skin bead-beating, or surface sampling with a flocked swab (n=2). c) Richness of untreated explant microbiota by sampling method (p=0.04). d) Shannon Diversity Index (richness and evenness) of untreated explant microbiota by sampling method (p=0.13). e) Boxplots of inverse Yue-Clayton Theta Index comparing the microbiome composition of explants within the same donor versus between donors. 1 represents 100% similarity, 0 complete dissimilarity (p = 0.006). f) Inverse Yue-Clayton Theta Index of similarity comparing the microbiome composition of explants processed on day 0 or at the end of the recommended experimental life of the explant (5–7 days) (p = 0.91). g) Mean relative abundance of endogenous NativeSkin flora by donor. 16S rRNA gene amplicon analysis, plotting the 15 most abundant species. N=2 for each donor/day. * indicates Kruskal Wallis chi-squared test p<0.05. **indicates bidirectional Wilcox rank sum test p < 0.05. NS (Not Significant).

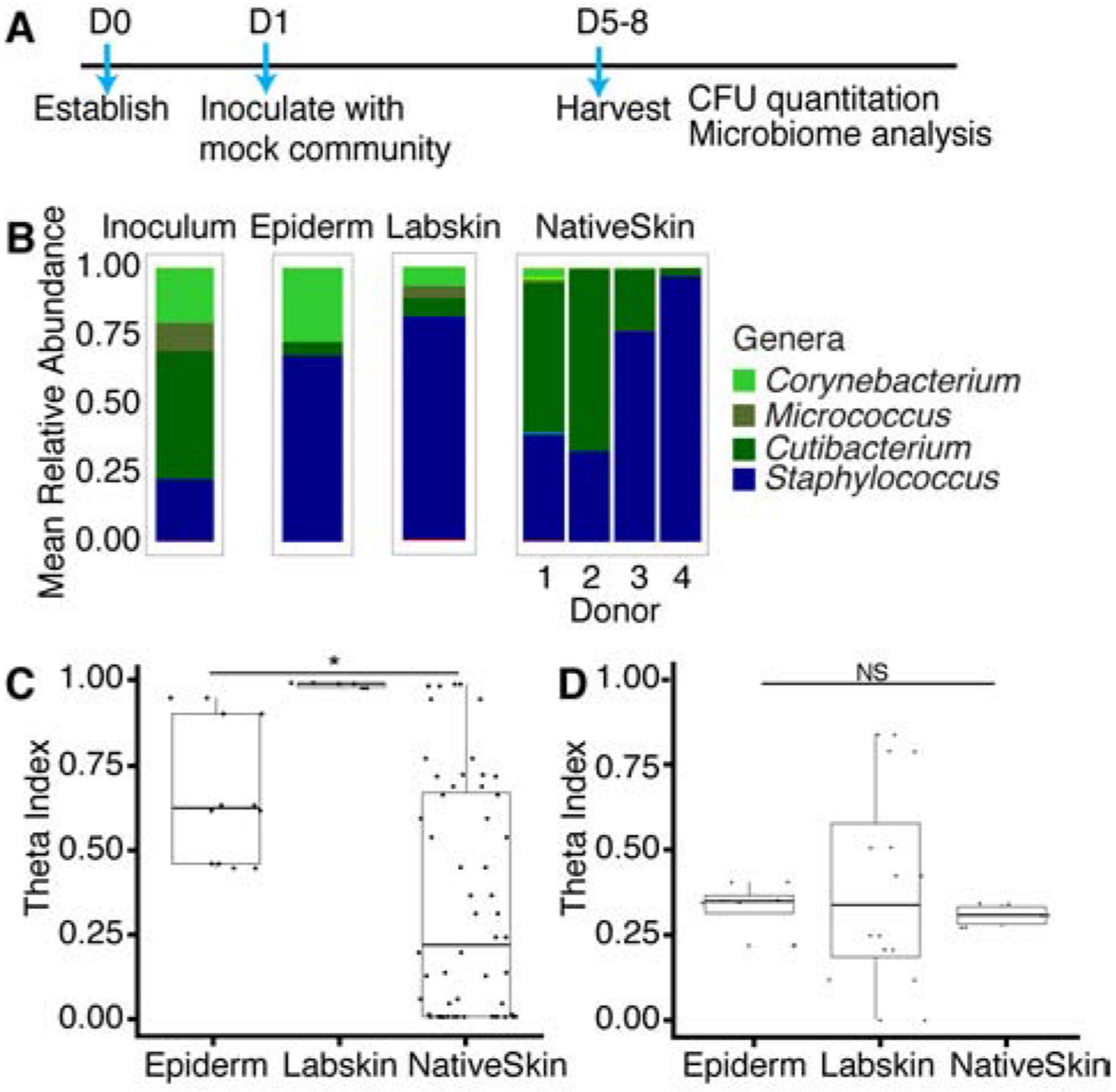

Figure 2:

Comparison of model systems for human skin microbiome experiments. a) Experimental timeline of colonization assays. b) Mock community culture on human skin models Mean relative abundance of 16S rRNA gene amplicon analysis, plotting the 15 most abundant species of a representative mock community inoculum, Epiderm (n =4) and Labskin (n = 3) RHEs, and NativeSkin explants (n=2 per donor). c) Inverse Yue-Clayton Theta Index of similarity comparing the composition of mock community cultured in replicate on each model system (p=2.6e-05). d) Inverse Yue-Clayton Theta Index of similarity comparing the composition of mock communities cultured on each model system to the original inoculum (p=0.81). * indicates Kruskal-Wallis H test $p<0.05$. NS (Not Significant).