Skin microbiota analysis in human 3D skin models—“Free your mice”

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Abstract
In the May issue of Experimental Dermatology 2018, we published a review article focusing on human 3D skin models in the context of microbiota research. The principal intention was to provide an overview of present and future concepts to use skin models in microbiota analyses. With the present viewpoint, we would like to draw the reader’s attention again to the use of human skin models in microbiota research with the aim to highlight the benefits and necessity of human skin models to analyse the human skin-microbiota interaction. This is accompanied by a critical view on mice models that often are not suitable to analyse the functional impact of the human skin microbiota. In addition, we present novel and future concepts highlighting the benefits of human 3D skin models in microbiota research.

KEYWORDS
3D skin equivalent, animal-free skin research, atopic dermatitis, keratinocytes, microbiome, skin microbiology

1 | WHY HUMAN SKIN MODELS?

There is rapid development in the generation of various human skin models. These encompass simple 2D models and more sophisticated 3D models including immunocompetent skin models. Human skin models offer one major advantage that should favour their future use: They are human! This simple statement may have important implications when investigating human skin-microbiota interactions. Many mouse models are in use to analyse skin physiology and biology, and it is beyond question that such mouse models help to address specific in vivo issues. However, mouse skin differs from human skin in many ways. In addition to differences in the anatomical structure (mouse skin is thinner due to fewer keratinocytes layers and contains more hair follicles), there are marked differences in gene expression. Gerber et al. discovered that there is only 30% identity between the top human- and mouse skin-associated genes.

The authors conclude that this huge diversity may explain why data generated in mouse models often fail to translate into humans.

There are also important differences in the composition of barrier-related structure proteins building the epidermal differentiation complex. Moreover, human skin harbours different subsets of immune cells and produces different cytokines as compared to mouse skin. Similarly, there are also distinct differences between skin-derived mouse and human antimicrobial peptides (AMPs), for example RNase 7 or SKALP (skin-derived antileukoprotease). This may have crucial consequences because AMPs are an important component of innate cutaneous defense, and there is increasing evidence that AMPs shape the microbiota and the microbiota in turn modulate AMP expression. For example, RNase 7 is a major human skin-derived AMP playing an important role in human cutaneous innate defense by controlling the growth of various microbes. Strikingly, an RNase 7 orthologue is not present in mice. As mice are more active...
under low light conditions, it is not surprising that also sunshine/UV-light-related modulation of gene expression differs between mice and man. For example, the expression of the human cathelicidin LL37 and the pattern recognition receptor NOD2 are induced in keratinocytes by the "sunshine" vitamin D3, whereas the respective mouse orthologues are not. It is very likely that such species-specific differences in innate cutaneous defense differentially influence the skin-microbiota interaction. This is also reflected by mouse-adapted bacterial strains and the uniqueness of the human skin microbiota within the class of Mammalia. In addition, the genetic background of mice, as well as the providers and rearing facilities, may have a profound influence on host-microbiota interactions and may affect the reproducibility of mouse microbiota studies and their conclusions.

Taken together, it is likely that the mouse skin evolved optimized mechanisms to shape and interact with its specific and preferred microbiota which differs clearly from the human skin microbiota. This in turn suggests that the mouse skin is not an adequate model to study the human skin-microbiota interaction and that mice experiments with human microbiota members likely result in misleading conclusions. Thus, we strongly encourage the reader to preferentially rely on data generated by human models rather than by murine models. This concern is also supported by the failure to translate the skin infection revealed by Rademacher et al. Given the huge heterogeneity and variance in the microbiota composition coupled with distinct host characteristics such as genetic factors or alterations in skin lipid metabolism. Dysregulation of lipid metabolism can lead to a compromised lipid envelope and thus an impaired barrier function, such as mutations in the gene encoding filaggrin (FLG), or alterations in skin lipid metabolism. Several pathophysiological mechanisms are thought to contribute to AD, but the two key drivers are a dysfunctional epidermal barrier and an exaggerated type 2 T helper cell (TH2)-mediated immune response. Multiple factors can contribute to an impaired barrier function, such as mutations in the gene encoding filaggrin (FLG), or alterations in skin lipid metabolism. More recently, shifts in the epidermal lipid composition have been reported to correlate with increased S aureus colonization. As the underlying factors that contribute to AD pathophysiology are complex, the identification of causative links remains a challenge, particularly when considering intradividual differences. Human 3D models have proved a valuable tool in getting one step closer to reveal causative factors influencing the skin barrier and microbial colonization. Mildner and colleagues showed that siRNA knockdown of FLG led to a disturbed barrier function in human skin models. Utilizing epidermal models, van Drongelen et al. showed that FLG knockdown generates novel hypotheses that need to be functionally verified in suitable models. Here, 3D models offer an unparalleled opportunity to study the human skin microbiota in a functional way. A comprehensive overview on different 3D human skin models is provided by Rademacher et al. Given the huge heterogeneity and variance of different methods to generate 3D skin models, it seems reasonable to define a set of standard parameters to validate the quality of the individual models. In this regard, van den Bogaard et al recently published a consensus paper suggesting several quality validation parameters based on morphological, biophysical and functional parameters. It is recommended to follow these principles in order to assess the suitability of the individual skin models for skin research.

3D skin models may represent a unique possibility to functionally analyse the relationship between biomedical and nutritional factors and their influence on the skin microbiota. Moreover, the use of siRNA to specifically knockdown genes of interest and the targeted overexpression of distinct genes provides an ideal tool in 3D models to simulate pathophysiological situations where a dysregulation of specific genes plays a role. Another option to study the influence of specific host factors on the skin-microbiota interaction may offer the generation of skin models with patient-derived keratinocytes that harbour intrinsic genetic aberrations such as loss-of-function mutations.

2 | ADVANCED 3D SKIN MODELS WILL PAVE THE WAY FOR FUNCTIONAL MICROBIOTA ANALYSES

In recent years, the prospect of exploiting the skin microbiome for skin health and personal care has attracted much interest. Recent studies have shown that the composition of skin microbial communities depends on various physiological and environmental factors. Skin localization is one of the main factors determining variance across microbial composition. Other biomedical and physiological factors contributing to skin microbiome variability include age, body mass index (BMI), sex and smoking. However, in-depth analysis of factors influencing the skin microbiome is difficult, as in vivo analysis of influencing factors in humans remains challenging. While recent years have seen a sharp increase in publications regarding skin microbiome changes related to biological, medical, environmental or even lifestyle factors, studies remain correlative and descriptive and cannot offer causative explanations due to a lack of suitable methods. Nevertheless, there is an increase in the number of studies performing a detailed comparative analysis of skin-microbiota composition coupled with distinct host characteristics such as genetic factors or transcriptomic signatures. Such studies
increased *S. aureus* colonization, thus proving the hypothesis that AD is associated with increased *S. aureus* colonization. An excellent summary of all FLG knockdown studies using 3D skin models is provided by Niehues et al. They showed that the transfer of bacteria from healthy volunteers but not from patients with AD improved outcomes of skin barrier disruption parameters in an AD-mouse model, which remains to be verified in human 3D models or patients. As discussed above, mouse models show marked differences in cutaneous immunity and microbiota biology compared to humans. Thus, it is out of question that studying the impact of the skin microbiota in dermatological diseases should be directly addressed in the human setup. Similarly, the use of 2D human tissue culture models to study AD is inherently doomed to fail, as they are per se not suitable to study epidermal 3D structure and cannot model the interactions of microbial communities with the differentiated human epithelium.

Although AD has been studied extensively, there are still many unanswered questions regarding which factors are responsible for the cutaneous microbiota dysregulation in the disease. Here, 3D skin models exposed to patient-derived microbiota may provide an ideal tool to uncover the responsible factors that contribute to skin microbiota changes in AD. For the future, it would be interesting to develop experimental approaches that allow to specifically label microbiota members derived from healthy and diseased skin to monitor microbiota-microbiota and microbiota-skin interactions on the surface of a 3D skin model. In addition, harmful pathogens and their skin and/or microbiota interactions could be easily investigated in such an approach without any ethical restrictions.

### 4 | 3D SKIN MODELS: SCAFFOLDS TO STUDY AND DEVELOP PROSPECTIVE THERAPEUTIC APPROACHES

Microbial colonization is driven by several environmental and lifestyle factors including BMI or obesity. While it is well established that dietary interventions can modulate the gut microbiota and possibly improve health, specific studies on skin health are lacking. Several independent studies have provided indirect evidence for the potential impact of diet on the human skin microbiome, including the finding of high microbial diversity in uncontacted Amerindians and association of the skin microbiome with BMI. A possible experimental approach to gain invaluable insight into the role of nutrient availability and its impact on skin microbiome composition utilizes labelled metabolites in a 3D skin microbiota model. In this model, labelled compounds could be added to the media and their metabolic conversion in fibroblasts, keratinocytes and microbiota detected by mass spectrometry. Given the heightened public interest in the modulation of the skin microbiome by systemic or nutritional approaches, the labelling of the compounds of interest and subsequent analysis of metabolization in the 3D skin microbiome model will enable researchers to answer questions about bioavailability of dietary supplementation in the skin.

Moreover, there is mounting confidence in the skin as a target for microbial products to control inflammation, mainly in the context of treating inflammatory skin disease. Baurecht et al. showed that the skin microbiome in AD is characterized by a shift from *Bacteroidetes* to *Staphylococcus* and is correlated with changes of lipids of the *stratum corneum*, highlighting that metabolic regulation of the skin microbiome is of importance for human skin health. First clinical pilot studies indicated that topical application of natural bacteria or probiotic solutions can improve skin barrier function and reduce AD severity, and multiple companies are developing topical microbiome-based skin therapies. Interestingly, there are first promising data available indicating that the microbiota of a recipient becomes more similar to the microbiota of a healthy donor. In this regard, it would be helpful to preselect specific microbiota compositions based on functional analyses to identify microbiota samples that elicit the desired effect. Here, functional experiments in 3D skin microbiota models will increase our knowledge on the direct and indirect mechanisms of modulating the skin microbiota, without having to rely on correlative but rather on functional data. Future research will hopefully enable us to apply our in-depth knowledge of the intricate mechanisms regulating the microbiota to develop cutting-edge personalized pre- and probiotic treatments.

### 5 | 3D SKIN MODELS AS HELPFUL TOOLS IN PERSONALIZED MEDICINE

3D skin models offer the advantage that they can also be constructed from patient-derived cells and thus have the potential to be utilized to study individual factors contributing to disease. In addition to invasive procedures, skin-derived cells can be elegantly obtained from hair follicle-derived keratinocytes, offering the possibility to construct personalized 3D skin models through a minimally invasive technique. It is also possible to generate pluripotent stem cells derived from hair follicles which opens the perspective to generate and investigate different cell types or organoids derived from the same individual. A further unique advantage of 3D skin models is that the method allows the autologous and heterologous transfer of microbiota. Standardized skin rinses from healthy individuals or patients can be transferred directly onto the 3D skin model, without the necessity to precultivate the microbiota. This approach can be additionally combined with patient-derived keratinocytes, thus representing a model where not only skin cells can be cultivated from patients, but also the microbiota (Figure 1). The additional incorporation of patient-derived fibroblasts and immune cells may lead to more sophisticated 3D skin models reflecting individual patient characteristics. Such models would allow to mimic the physiological cross-talk of the epidermis and dermis including involved immune cells. Moreover, the use of such individualized models will allow us to precisely dissect the individual impact of the host cells and its microbiota for disease development. However, the incorporation of immune cells into 3D models remains a challenge due to different media requirements.
Additionally, the even seeding of immune cells as well as successful migration of immune cells into the 3D models continues to pose a challenge.\textsuperscript{[51]} Despite the difficulties, a few groups have been successful in incorporating immune cells into 3D skin models, thus presenting a model to study inflammatory skin diseases.\textsuperscript{[52-54]} Beside immune cells, incorporation of skin appendages, for example hair follicles or sebaceous gland, is of considerable interest, but research is still in its infancy. A recent study from Lee et al\textsuperscript{[55]} described a method to construct a hair-bearing skin organoid culture system that can be used to reconstitute skin in vivo. We believe that the future development of these complex skin models will offer ingenious perspectives; however, at the moment these complex skin models will probably remain a second-line research method due to the time-expensive incubation of 4-5 months necessary for successful construction. Further incorporation of novel high-throughput methods like metagenome or whole transcriptome sequencing of microbiota-exposed individualized 3D skin models will prove an additional advantage to unravel the significance of a dysregulated cutaneous host-microbiota interaction. Taken together, 3D skin models consisting of patient-derived cells and microbiota could represent a cutting-edge technique to develop and study targeted personalized interventions of the skin microbiota (Figure 2).

Clearly, the more the 3D skin models can reflect the in vivo situation, the better the successful translation of the findings into medical treatment options will be. In this regard, full-thickness human skin-on-chip models may provide a future sophisticated...
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system to mimic the 3D microenvironments of human skin in a more standardized and high-throughput manner. In these models, a microfluid system allows for specifically adjusting the supply and/or drainage of nutrients and metabolites. Full-thickness complex human skin models generated by a 3D cell-printing process may offer another feasible strategy to reflect the complexity of native human skin. Of note, such vascularized skin models represent a further step forward to simulate the in vivo situation. In this context, 3D bioprinting using specific bioink offers the possibility to generate biomimetic skin constructs in a fast and reproducible way because the automated process facilitates the deposition of specific cell types at desired positions. It is intriguing to speculate that the combination of 3D bioprinting with patient-based bioink (including individual microbiota compositions) will allow for the construction of an in vitro copy of an individual diseased skin state. In the light of these recent developments, it will be exciting to see how future research will unravel personalized models to study individual pathophysiological factors contributing to different skin diseases.

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FIGURE 2 Shown is the suggested scenario to develop an individualized microbiota-based treatment approach. This includes a functional analysis using a 3D skin model generated with patient-derived hair keratinocytes and consecutive treatment of the 3D skin model with the patient-derived microbiota. This would allow to develop the most profitable, personalized treatment with a patient-designed beneficial microbiota accompanied by a minimized risk of eg side effects.

CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTIONS

HE, FR and JH wrote the manuscript. RG and JH critically revised the manuscript.

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