# Toward engineering the human skin ecosystem: integrating skin microbiota, sweat, and organ-on-chip technology

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## Abstract

Current skin microbiota models are limited in their ability to mimic the complexity of healthy human skin. Here, we developed a physiologically relevant full-thickness skin-on-chip model integrating commensal microbiota and artificial sweat, providing a novel platform to study microbiota dynamics and skin barrier function.

## Introduction

The skin microbiota constitutes a highly dynamic ecosystem that plays a crucial role in maintaining skin homeostasis and barrier function. Commensal microorganisms contribute to pathogen exclusion, immune modulation, and the regulation of epidermal differentiation, while their imbalance has been associated with multiple skin disorders (Byrd, Belkaid, and Segre 2018; Grice and Segre 2011).

Despite the increasing interest in studying skin microbiota, most currently available in vitro models mainly focus on pathological conditions and frequently fail to reproduce the complexity of the healthy skin microenvironment. In many cases, these models rely on the incorporation of single microorganisms or simplified skin structures, limiting their physiological relevance and their ability to reproduce native host–microbiota interactions (Baroni et al. 2004; Kılıç et al. 2019; Rikken et al. 2023). Furthermore, the influence of key skin microenvironment components, such as sweat, remains poorly explored, despite its important role in regulating nutrient availability, osmolarity, pH, and microbial growth on the skin surface (Baker 2019; Swaney et al. 2023).

Therefore, the aim of this work was to develop a physiologically relevant full-thickness skin-on-chip model integrating representative commensal skin microbiota and artificial sweat to study microbiota behaviour, skin barrier integrity, and tissue–microbiota interactions under both healthy and pathological skin conditions.

## Materials and Methods

We characterized and quantified by qPCR four representative commensal bacterial strains, *Staphylococcus capitis*, *Staphylococcus hominis*, *Corynebacterium afermentans*, and *Corynebacterium simulans*, in order to simulate a realistic skin microbiota pool and enable the specific detection of each strain at low abundances.

A full-thickness skin-on-chip model was established using a BE-Transflow microfluidic device, consisting of a dermal compartment formed by fibrin-based hydrogels with embedded human dermal fibroblasts and a stratified and cornified epidermal layer generated using immortalized human keratinocytes.

Bacterial pool inoculation was performed on day 14, once full epidermal stratification had occurred. For inoculation, bacterial pools were resuspended either in PBS or artificial sweat. Samples were collected from the apical compartment of the model and from microdevice effluents at different time points to quantify each bacterial strain under both conditions and evaluate bacterial dynamics over time. Finally, the same procedure was performed using skin-on-chip models with disrupted epidermal barrier integrity.

# Results

Representative commensal bacterial strains commonly found in moist skin areas, including *S.capitis*, *S.hominis*, *C.afermentans*, and *C.simulans*, were successfully characterized and quantified. Growth kinetics and qPCR standard curves demonstrated high sensitivity and specificity for the detection of each strain at low bacterial abundances.

The influence of sweat on microbiota dynamics was evaluated by resuspending bacterial pools in PBS or artificial sweat. Overall, bacterial abundance decreased more markedly under sweat conditions compared with PBS, particularly in *S. capitis*, *S. hominis*, and *C. simulans* strains, reached stable population levels over time. These findings suggest that sweat contributes to maintaining microbiota stability under physiologically relevant conditions.

Histological analysis confirmed proper epidermal differentiation and stratification of full-thickness skin-on-chip model (Figure 1). Following microbiota inoculation in either PBS or sweat conditions, bacterial populations remained localized on the epidermal surface without detectable translocation into the microfluidic channels, demonstrating effective barrier integrity and model stability.

Finally, disruption of the epidermal barrier altered microbiota dynamics and promoted bacterial translocation behavior. While *S. hominis* maintained stable colonization on the epidermal surface, *Corynebacterium* strains progressively disappeared from the epidermal compartment after barrier disruption, suggesting migration through the damaged tissue. These findings highlight the importance of skin barrier integrity in maintaining microbiota homeostasis and preventing opportunistic bacterial behavior.

# Conclusions

The integration of sweat into full-thickness skin-on-chip models contributes to maintaining microbiota stability and reproducing a more physiologically relevant skin microenvironment. In addition, epidermal barrier disruption alters microbiota dynamics and promotes bacterial translocation, highlighting the critical role of skin barrier integrity in maintaining microbial homeostasis. These findings have important implications for the development of advanced skin microbiota models, enabling the generation of more realistic platforms for studying host–microbiota interactions and evaluating pharmaceutical and cosmetic compounds.

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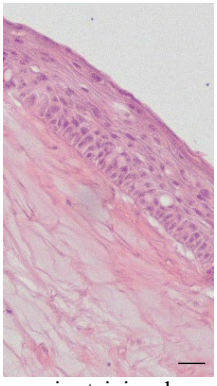

**Figure 1.** Haematoxylin–eosin staining demostrating proper full-thickness skin-on-chip formation and epidermal stratification. Scale bar: 50 μm.