INTRODUCTION

3D skin models are getting closer to the reality of cutaneous physiology. They can include multiple cell types, from healthy or pathological skin or be submitted to various stresses mimicking the skin’s environment. But most of these models are sterile. In recent years, skin microbiota has emerged as a key player in skin health, preventing pathogens proliferation, educating the immune system and maintaining barrier integrity. The first skin models colonized with bacteria have focused on unique bacterial species. But the study of skin diseases (such as atopic dermatitis) revealed that microbiota’s diversity is key to skin health. We thus developed a model that reproduces the complexity of the skin’s ecosystem with an uncultered skin microbiota and compared it to the use of a unique commensal bacteria.

METHODS

Skin microbiota (SM) was collected from the inner forearm with nylon swabs and then retrieved, centrifuged and resuspended in the appropriate volume. Labskin models (Innovenn Ltd) were inoculated with bacterial suspension (20 µl) equivalent to 1 cm² of collected SM or 10⁴ CFU of ATCC12228 Staphylococcus epidermidis. Bacteria were incubated at 37°C-5% CO₂ for 4h to 7 days.

RESULTS

QPCR standard curve correlates cycles (Cq) and Colony-Forming Unit (CFU)

- 16S rRNA gene

Genomic DNA was extracted from known numbers of SE CFU and used as a template for QPCR with universal 16S rRNA. Here, we show that the Cq values obtained are correlated with the CFU numbers. We used this standard curve to calculate the number of bacteria present on skin models by CFU equivalent (CFU eq.) at different time points.

CONCLUSION

Here we inoculated labskins with an unselected skin microbiota containing the bacteria, viruses, yeasts and fungi that compose skin’s ecosystem. We observed that besides growing slower than the unique bacterial species S.epidermidis, it also dramatically increased epidermal proliferation and cohesion. Other skin markers should also be analyzed to better understand the relationship between skin and commensal bacteria. Such model could show the benefits of preserving a healthy and viable microbiota and its possible modifications by stress or product application.

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Skin microbiota is better tolerated than S. epidermidis

At day 7, the rapid growth of SE led to a disorganized epidermis associated with decreased cell junctions and pyocytic nail retention in the SC (ectar). On the contrary, SM induced a more proliferative basal layer (Ki67) and a higher cell cohesion as shown by tight junctions (claudin 1; CLDN1), desmosomes (desmoglein 1; DSG1) and corneodesmosomes (corneodesmosine; CNDSN) proteins. In both SE or SM-inoculated skin, the SC presented a surprising persistence of these junctional proteins (arrows) which could reflect an accelerated desquamation process in response to bacteria. The dotted line underlines the dermal-epidermal junction.