COLONIZATION OF A 3D SKIN MODEL WITH A COMPLETE MICROBIOTA IS MORE BENEFICIAL TO THE SKIN BARRIER THAN WITH *STAPHYLOCOCCUS EPIDERMIDIS* ALONE

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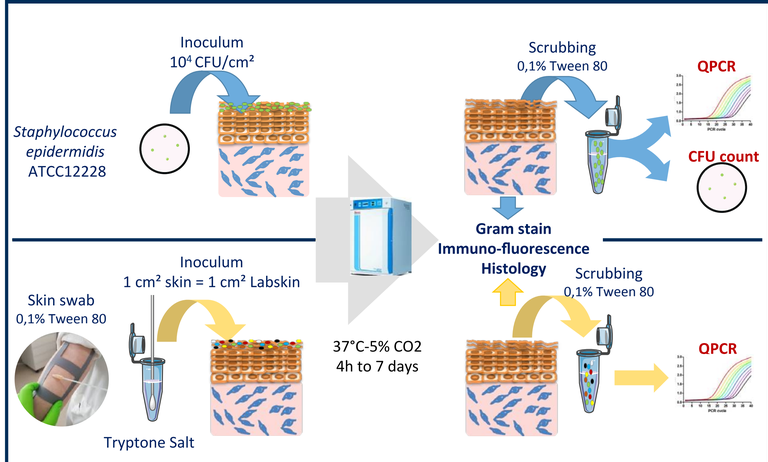
INTRODUCTION

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 uncultured skin microbiota and compared it to the use of a unique commensal bacteria.

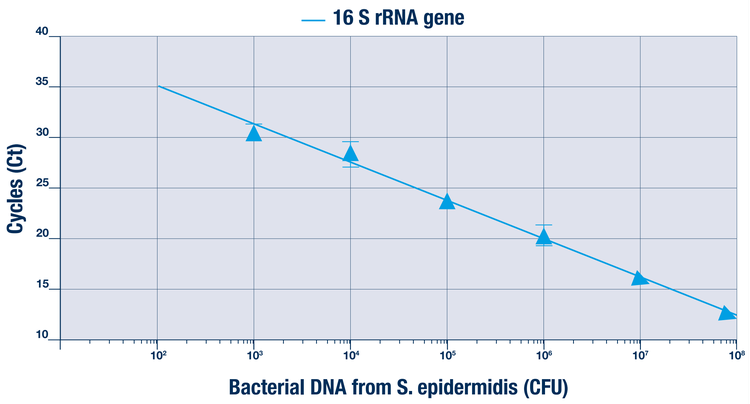
METHODS



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 Staphylococcus epidermidis (SE). After 4 hours to 7 days in culture, some skin models were gently scrubbed with a sterile syringe for bacteria count by colony forming unit (CFU) count and/or real-time PCR QPCR (n=3). At day 7, some skin models were collected for histology (n=3).

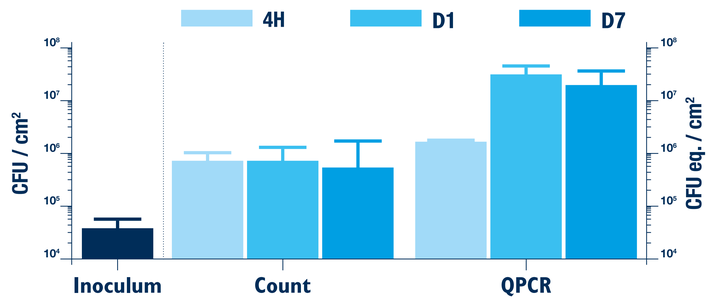
RESULTS

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



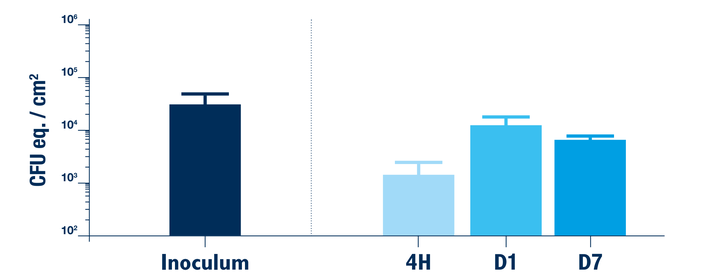
Genomic DNA was extracted from known numbers of SE CFU and used as a template for QPCR with universal 16S rRNA. Here, we show that the Ct values obtained are correlated with the CFU numbers. We used this standard curve to calculate the number of bacteria present on skin models by CFU equivalent (CFU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

While *S. epidermidis* rapidly grows on the skin surface…



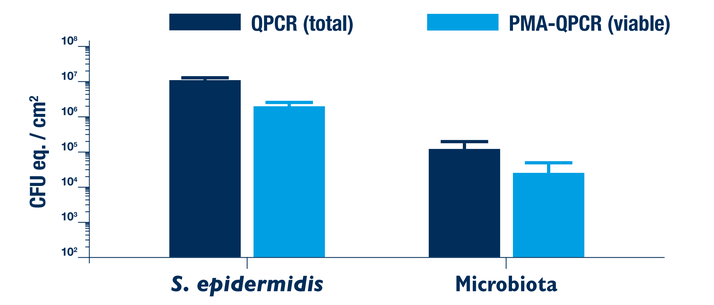
While the number of viable **SE** grew up to nearly 10^6 CFU/cm² 4 hours after inoculation and then stabilized from Day 1, the total number of **SE** detected by QPCR was 25 times higher (2.10^7 CFU eq./cm²), suggesting the **persistence of dead bacteria on the stratum corneum** (SC) in the absence of corneocytes elimination in culture.

… Skin microbiota stays stable



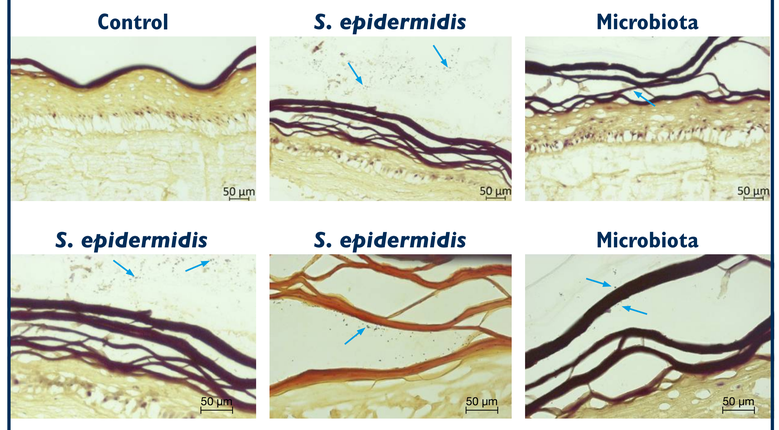
Shortly after **SM** inoculation (4.10^4 CFU eq./cm²), a slight decrease was observed after 4h incubation (4.10^3 CFU eq./cm²). This observation reflects an incomplete retrieval of bacteria that could result from insufficient scrubbing and/or to their penetration into the skin layers. Then, after a growth, microbiota density stabilized between day 1 and day 7.

Skin microbiota is alive and stabilized at physiological density



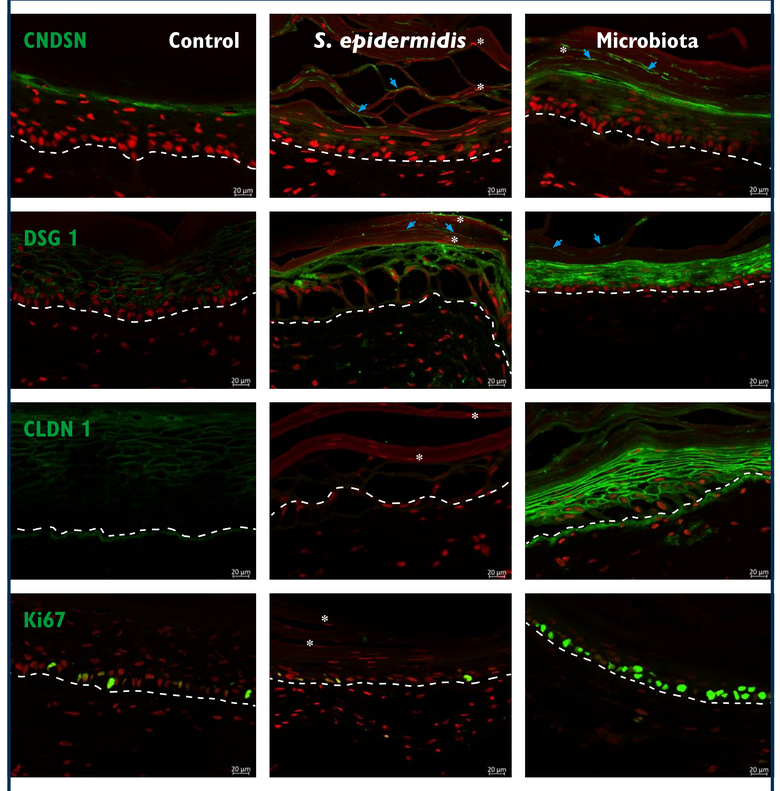
While QPCR amplifies gDNA from viable and dead bacteria, PMA-QPCR allows the selective detection of viable bacteria. PMA dye penetrates the compromised membrane of dead bacteria and, when exposed to light, binds covalently to gDNA, avoiding its amplification. In this experiment, 5 days after inoculation, results confirm that **viable-SE** grew up to a non physiological density (2.10^6 CFU eq./cm²) while **viable-SM** density (2.10^4 CFU eq./cm²) remained equivalent to that found on the donor skin (4.10^4 CFU eq./cm²). In absence of skin washing and desquamation, dead bacteria accumulation did not impact the number of viable ones.

Skin microbiota is better tolerated than *S. epidermidis*



Gram stain at day 7 confirmed a higher number of bacteria on the epidermis inoculated with **SE (arrows)** compared to **SM** (Gram-positive: purple). Epidermis with SE was thinner and disorganized while **SM** did not seem to affect epidermis integrity **(upper panel)**. Both **SE** and **SM** induced more SC layers compared to the control. Interestingly, some bacteria penetrated in between the SC layers but not into the living epidermis **(lower panel)**.

Skin microbiota increases epidermal renewal and keratinocytes cohesion



At day 7, the rapid growth of **SE** led to a disorganized epidermis associated with decreased cell junctions and pycnotic nuclei retention in the SC **(stars)**. 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.

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.

ACKNOWLEDGEMENTS

We'd like to thank David Buiguez and Benoit Havel from the L'Occitane microbiology team for their help on bacterial culture; Malvina Olivero, Claire Jousselin and Maelys Devincenzi for their help on histology and confocal microscopy.

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