The Key role of *S. epidermidis* abundance in human skin differentiation and inflammation

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Abstract

Background: *Staphylococcus epidermidis* is a commensal bacterium ubiquitously present on human skin[1], reported to be involved in the defense against pathogens[2], the modulation of the immune system and the wound repair[3]. However, an overgrowth of *S. epidermidis* has been described in skin disorders such as atopic dermatitis[4] where it induces protease activity leading to skin barrier damages and inflammation[5]. Thereby we sought to understand the impact of *S. epidermidis* abundance on inflammation and epidermal differentiation markers of normal human skin.

Methods: A low (10^3 UFC/cm²) or a high (10^6 UFC/cm²) dose of *S. epidermidis* ATCC 12228 were inoculated on a reconstructed human skin model mimicking a healthy and disease states respectively. Their impact on epidermal differentiation has been studied by transcriptomic, histological analyses and measurement of cytokines secretion levels until 7 days of colonization.

Results: We showed that a high dose of *S. epidermidis* on the skin surface, significantly reduces the living epidermis thickness and modulates biological pathways implicated in inflammatory response, and skin development; specifically the differentiation of the epidermis was altered at the gene expression and protein levels. Conversely, a low dose of bacteria does not affect the epidermis structure, nor inflammation and the epidermal differentiation complex genes (EDC) are preserved compared to the controls.

Conclusion: This study shows a first direct link between *S. epidermidis* abundance and epidermis structure, differentiation, and inflammation context. All together, these data allow us to develop a healthy human skin model colonized by bacteria and emphasize the importance of the good balance of *S. epidermidis* quantity for healthy skin quality.

Keywords: cutaneous microbiota; epidermic differentiation; microorganisms/skin interaction models; reconstructed skin

Introduction.

The role of bacterial gut microbiota has been extensively investigated in the last decade, and now more and more studies describe the microbiota of other niches. Thus, the bacterial skin microbiota has been recently precisely characterized[1]. Its influence on skin health and skin development is likely but not yet clearly proven and deciphered. Elucidating the effect of skin commensals directly on skin is now a key path to better understand the role of the skin microbiota in skin health and disease.

Staphylococcus epidermidis is a coagulase negative commensal bacterium is generally considered as a key member of healthy skin microbiota, involved in the defense against pathogens[2], the modulation of the immune system and the wound repair[3].

Simultaneously, *S. epidermidis* is the second cause of nosocomial infections and an overgrowth of *S. epidermidis* has been described in skin disorders such as atopic dermatitis[4] and induces protease activity leading to skin barrier damages and inflammation[5] in a mouse model.

Recently, human skin equivalents models have been used as powerful tool showing pronounced changes in the transcriptional profiles of the skin in response to the presence of a microbial community or a microbe at a single concentration[6].

Thereby, in this study, we sought to understand the impact of *S. epidermidis* abundance on inflammation and epidermal barrier using a reconstructed human skin model. For this purpose, reconstructed skins were inoculated with a low (10^3 UFC/cm²) or a high (10^6 UFC/cm²) load of *S. epidermidis* ATCC 12228 mimicking a healthy and disease states respectively. The impact of bacterial abundance on epidermal differentiation were studied by

transcriptomic, histological analyses and measurement of cytokines secretion levels during 7 days of colonization.

Materials and Methods.

Bacteria and skin model co-culture:

LabSkin models (Innovenn Ltd) were treated with a high (10^6 UFC/cm²) or low (10^3 UFC/cm²) inoculum of *Staphylococcus epidermidis* ATCC 12228 prepared in 20 µL of medium (Fig. 1). After 1, 2 and 5 days of treatment, samples were gently swabbed to remove bacteria and CFU were counted on agar plate. Skin tissues were collected, half for histological and a half for transcriptome analyzes. Culture media were collected for cytokines quantification.

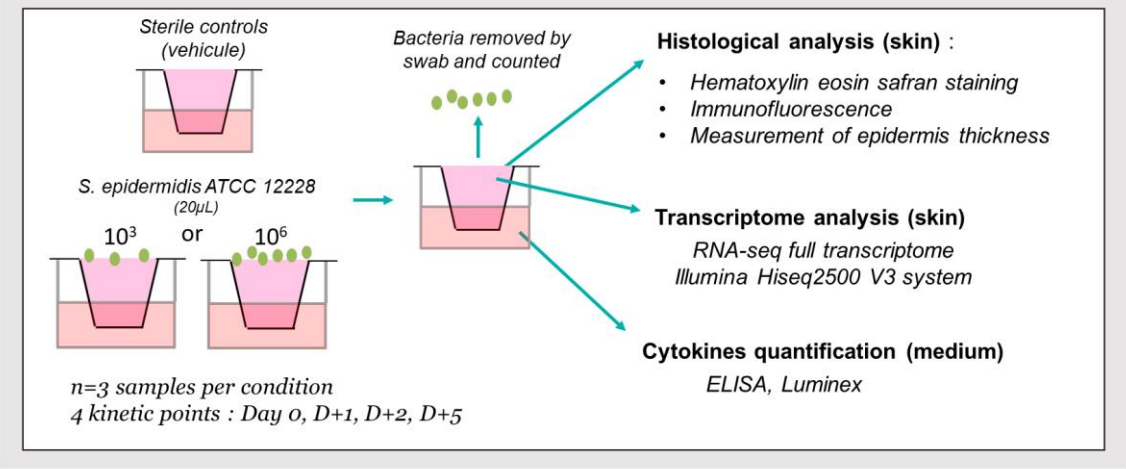

Figure 1: Schematic representation of the experiment. Reconstructed human skin models LabSkin were treated with S. epidermidis ATCC 12228 with a high inoculum of 10^6 UFC/cm² or a low inoculum of 10^3 UFC/cm². Sterile controls were treated with the vehicule. Samples were collected at Day 0, D+1, D+2, D+5 post treatment. The culture media were collected for cytokine quantification; LabSkin were swabbed to remove the bacteria, half was used for histological analysis and half was used for transcriptome analysis using Novaseq 6000 platform.

Histological analyses:

Tissues were conserved in OCT at -20°C, stained with hematoxylin eosin safran and the epidermis thickness was measured (≈600 measurements/tissue). Immunofluorescence labbeling was used to highlight the protein expression of differentiation markers.

Cytokines measurement:

Cytokines were quantified in culture media with Quantikine ELISA kit (R&D) or Milliplex MAP kit (Millipore) according to the manufacturer instructions.

Transcriptome analyzes:

RNAs were extracted and purified using the RNeasy Mini Kit (Qiagen, 74104) according to the supplier's instruction. Library were obtained after sequencing on an Illumina Hiseq2500 V3 system. Data were normalized with Kallisto software and analyzed with FactoMineR for ACP and DESeq2 for differential expression analyzes. Statistical analyzes were performed with R.

Results.

The bacteria are viable for 7 days on LabSkin model:

To evaluate the influence of the bacterial abundance on the skin, we developed co-culture models. Therefore, the reconstructed skin models LabSkin have been inoculated by 10^3 or 10^6 CFU/cm². We first verified the viability of the bacteria on the skin model surface. We observed that *S. epidermidis* can colonize the LabSkin model either with 10^3 or 10^6 CFU/cm²: from 10^3 to 10^6 UFC/cm² and 10^6 to 10^8 UFC/cm² respectively (Fig. 2). These models are viable for 7 days.

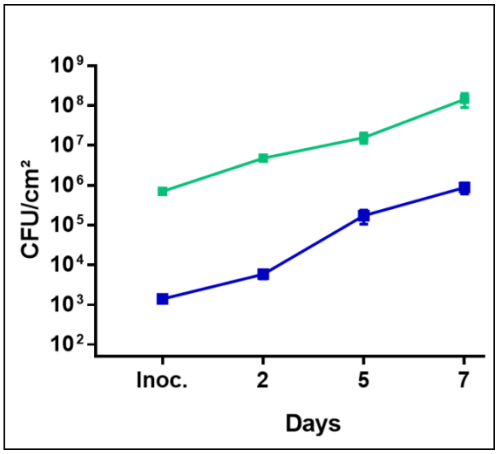

Figure 2: S. epidermidis growth on LabSkin tissues with 10^3 (blue line) or 10^6 (green line) bacteria inoculated. CFU: colony forming

Epidermis thickness depends on *S. epidermidis* inoculum:

The epidermis structure of tissues inoculated with a high inoculum is altered whereas those inoculated with a low inoculum are comparable to the control despite the bacterial growth (Fig.2). Particularly, with a high inoculum, the living layers of the epidermis are significantly thinner compared to the control (Fig. 3; Fig.4) at day+5 and day+7 post inoculum.

These results confirm existing report by Loomis et al.[6] where they inoculated with 5.10^5 CFU/cm² for 5 days the EpiDerm model (MatTek) and expand their finding with a longer kinetic.

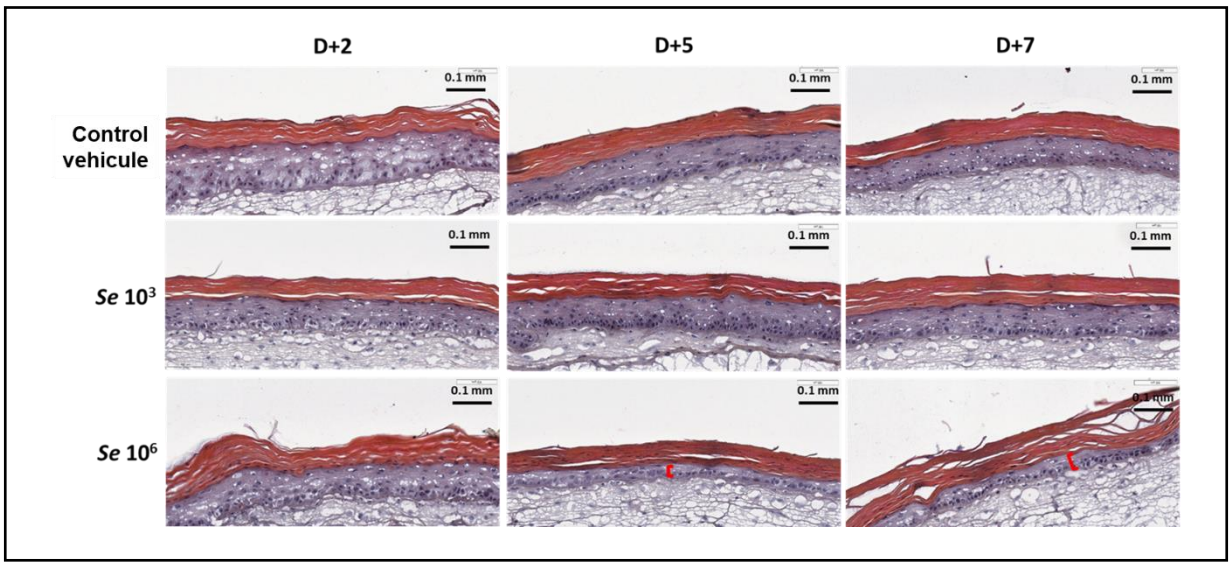

Figure 3: Hematoxylin eosin safran staining of LabSkin tissues inoculated with a low dose of 10^3 or a high dose of 10^6 of S. epidermidis (Se) at day+2, +5 and +7 post treatment. Sterile controls were inoculated by vehicule.

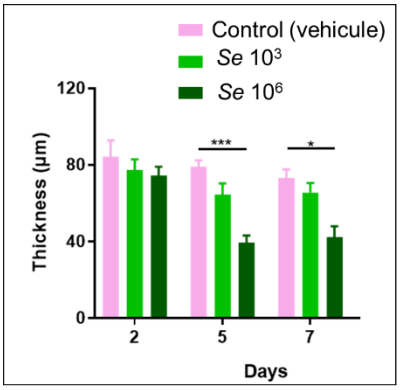

Figure 4: Thickness measurements of living layers of epidermidis. Measurements were conducted on each sample and averaged per group of treatment.

Inflammation depends on *S. epidermidis* inoculum :

At the gene expression level, the tissues inoculated with 10^6 CFU/cm² showed significantly higher expression of inflammation markers compared to the sterile control (Fig.5) during all the kinetic. This inflammation context was confirmed at the protein level (Fig.6). On the contrary, the tissues inoculated with 10^3 CFU/cm² are comparable to the control at D+1 and D+2 at the gene and protein expression level. At D+5, inflammation genes expression increases but that was not confirmed at the protein secretion level indicating that the low inoculum do not induce inflammation.

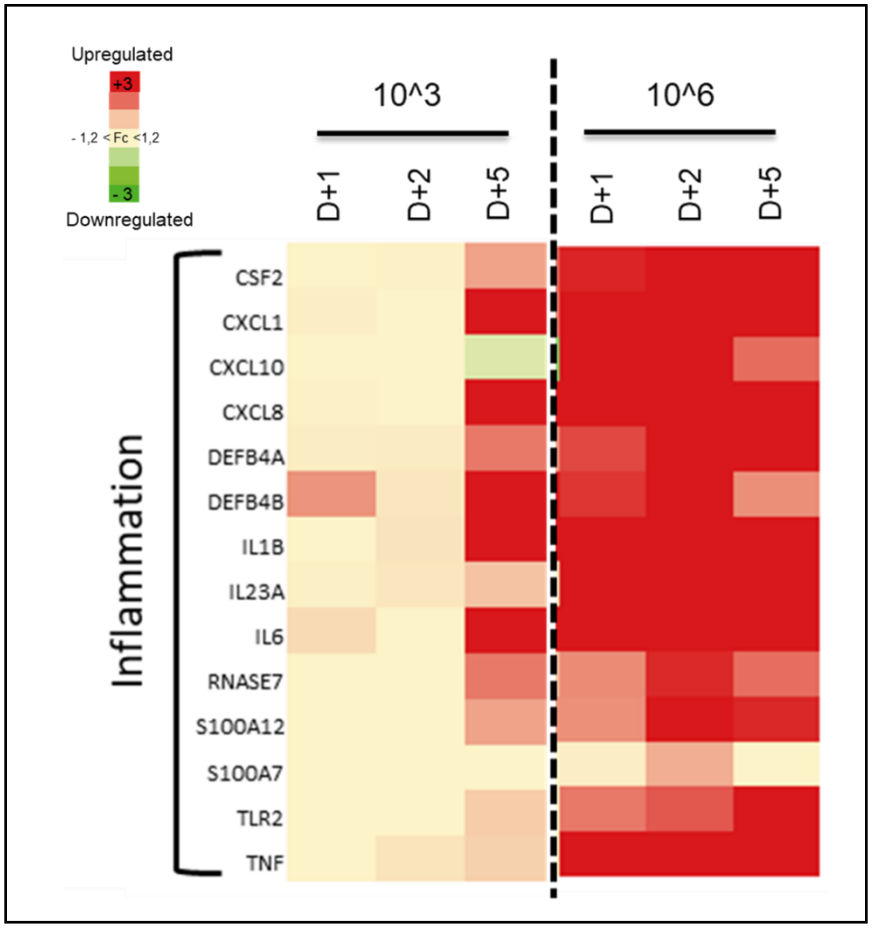

Figure 5: Heatmap representing the expression of inflammation markers after S. epidermidis colonization with 10^3 or 10^6 CFU/cm² compared to the sterile control. Foldchange significatively upregulated are indicated in red whereas those downregulated are in green.

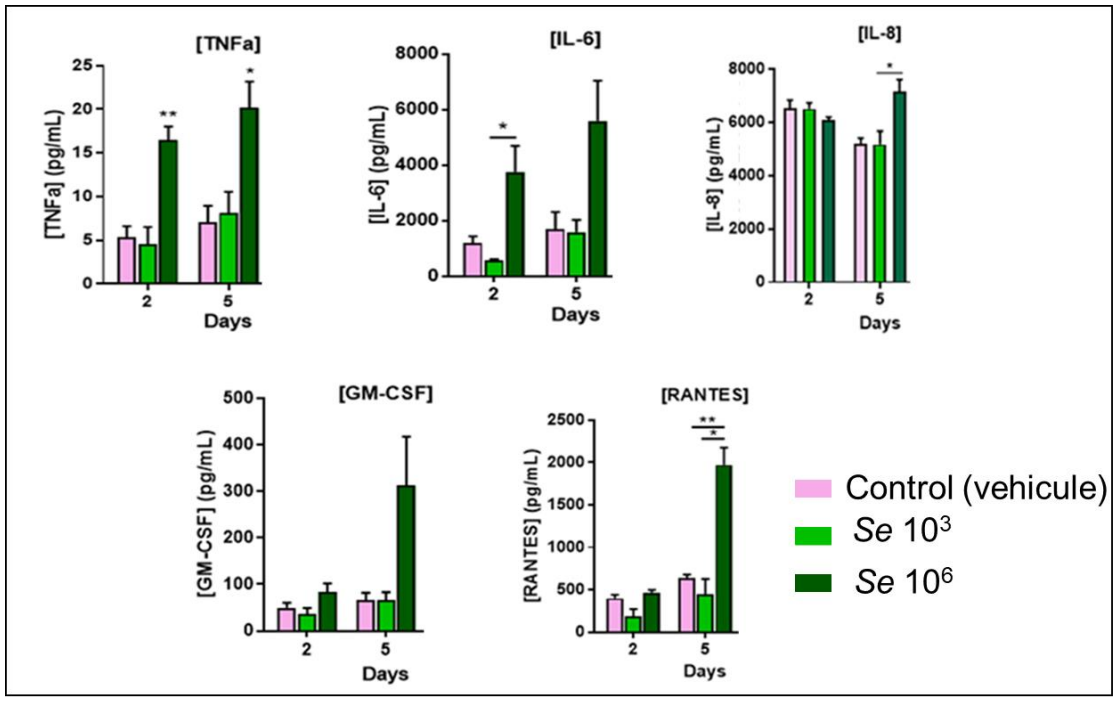

Figure 6: Quantification of TNFa, IL-6, IL-8, GM-CSF (CSF2), and RANTES in the culture conditioned media of sterile controls or LabSkin tissues inoculated with S. epidermidis at a low dose of 10^3 CFU/cm² or a high dose of 10^6 CFU/cm². Quantifications were conducted on individual samples and averaged for each condition.

Epidermis differentiation is modulated by *S. epidermidis* inoculum:

Transcriptome analysis showed that epidermis differentiation is one of the most modulated metabolic pathways.

Tissues inoculated with 10^6 CFU/cm² showed a significant downregulation of skin differentiation genes expression and was confirmed at the protein level, for filaggrin as an example, by immunofluorescence staining (Fig. 7). Conversely, with a low inoculum, there is a weaker and delayed decrease of skin differentiation genes expression, that is not expressed at the protein level, indeed the filaggrin protein expression is comparable to the control for the tissues inoculated with 10^3 CFU/cm².

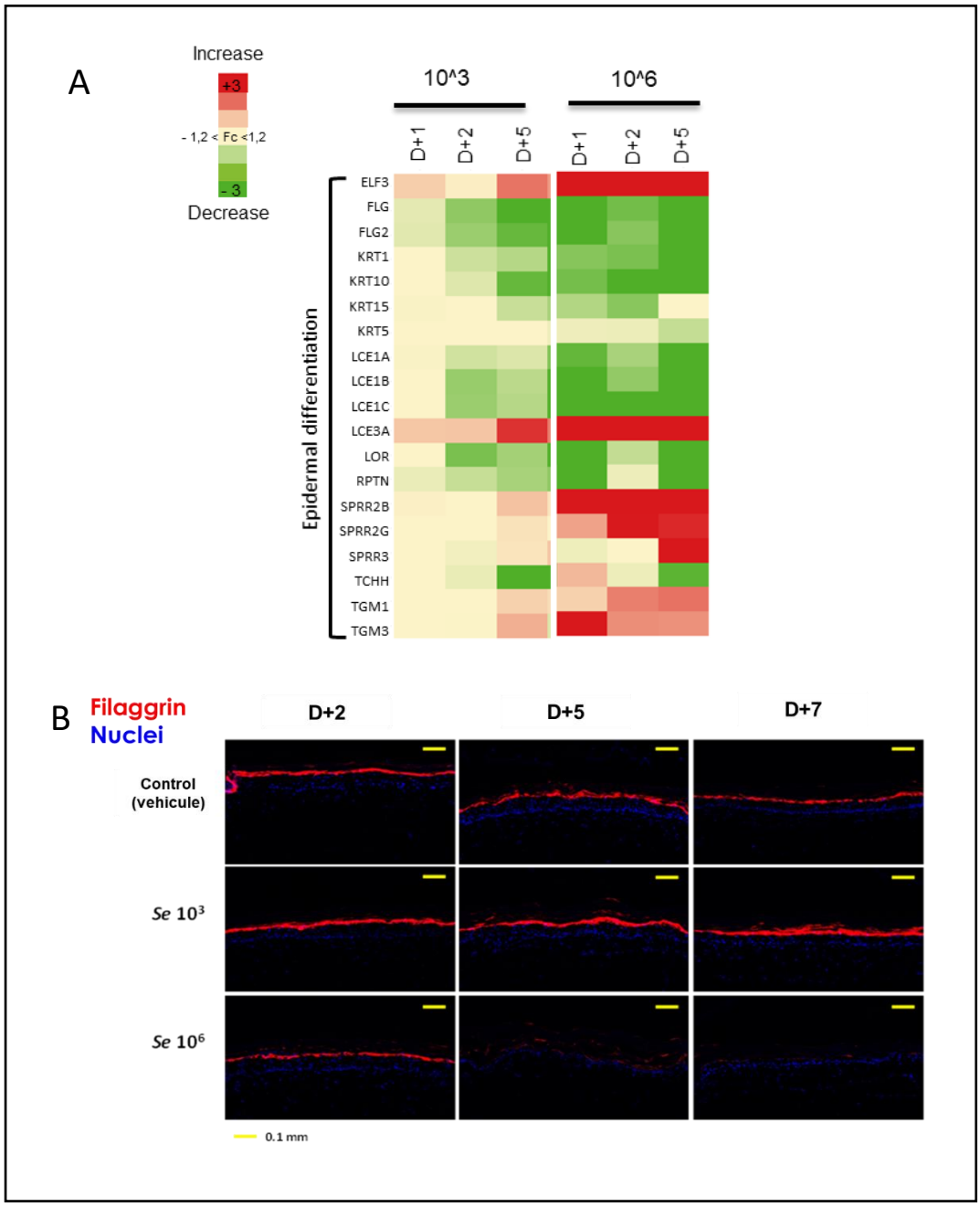

Figure 7: (A) Heatmap representing the expression of differentiation markers after S. epidermidis colonization with 10^3 or 10^6 CFU/cm² compared to the sterile control. Foldchange significatively upregulated are indicated in red whereas those downregulated are in green. (B) Immunofluorescence staining of the filaggrin protein (red) and nuclei (blue) for LabSkin tissues inoculated with a low dose of 10^3 or a high dose of 10^6 of S. epidermidis (Se) at day+2, +5 and +7 post treatment. Sterile controls were inoculated by vehicule.

Discussion.

Currently, there are few 3D models colonized by bacteria described in the literature. Most of them have been designed to study skin disorders such atopic dermatitis, psoriasis or acne[7-11]. Therefore, these 3D models were inoculated by bacteria considered as pathogenic, with a high dose inoculated, during a short contact time. The reported models today mimicking a healthy skin state are generally inoculated with *Staphylococcus epidermidis* but with a high inoculum (10^5 to 10^9 UFC/cm²) whereas a recent study showed in a mouse model that the overabundance of *S. epidermidis* (above 10^6 UFC/cm²) induces protease activity leading to skin barrier damages and inflammation[5]. Thus, to clarify the influence of *S. epidermidis* abundance on skin, we developed two new models with a low or high inoculum of 10^3 and 10^5 UFC/cm² respectively. Compared to published colonized skin models, we showed for the first time that *S. epidermidis* abundance influence epidermis structure, inflammation, and differentiation and must be considered to develop a healthy skin model. A high inoculum induces a significant thinning of the living layers of the epidermis, decrease the differentiation markers at the molecular and protein levels and increase inflammation while 3D models inoculated with a low inoculum of the same *S. epidermidis* strain is comparable to the control.

Hence, we developed a new simple healthy colonized human skin model, viable for 7 days which is a longer kinetics than the existing models, that is more convenient for cosmetic evaluation.

Conclusion.

This study shows a first link between *S. epidermidis* abundance and epidermis structure, differentiation and inflammation context. All together, these data allow us to develop a simple healthy colonized human skin model. Further *in vitro* studies will be needed including other microbial species of the skin microbiome, various skin models/donors and a more comprehensive *in vivo* analysis of the abundance of *S. epidermidis* on healthy or altered skin. However, this constitutes a major step in the understanding of the importance of the good balance of *S. epidermidis* quantity for healthy skin quality.

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Conflict of Interest Statement.

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