THE KEY ROLE OF *S. EPIDERMIDIS* ABUNDANCE IN HUMAN SKIN DIFFERENTIATION AND INFLAMMATION

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Staphylococcus epidermidis is a commensal bacterium ubiquitously present on human skin[1]. This species is generally considered as a key member of healthy skin microbiota, described 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] and a recent study showed in a mouse model that abundant *S. epidermidis* induces protease activity leading to skin barrier damages and inflammation[5].
Nevertheless, little is known about the impact of *S. epidermidis* abundance on inflammation and epidermal differentiation markers on normal human skin.
For this purpose, we inoculated either a low (10^3 UFC/cm²) or a high (10^6 UFC/cm²) inoculum of *S. epidermidis* on a reconstructed human skin model and compared their impact on epidermal barrier by histological analyzes, measurement of cytokines secretion and transcriptome analyses.

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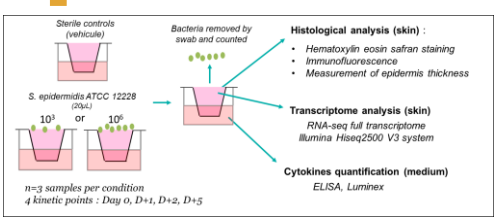

Figure 1: Schematic representation of the experiment. Reconstructed human skin models LabSkin were treated with S. epidermidis ATCC 12228 with a high inoculum of 10^6 UFC/cm² or a low inoculum of 10^3 UFC/cm². Sterile controls were treated with the vehicule. Samples were collected at Day 0, D+1, D+2, 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.

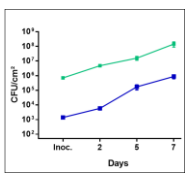

Figure 2: S. epidermidis growth on LabSkin tissues with 10^3 or 10^6 bacteria inoculated. CFU : colony forming unit

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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.3). 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 5.105 CFU/cm² for 5 days the EpiDerm model (MatTek) and these results expand their finding with a longer kinetic.

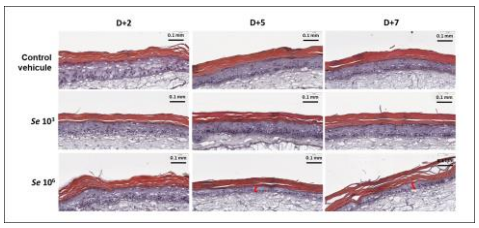

Figure 3: Hematoxylin eosin safran staining of LabSkin tissues inoculated with a low dose of 10^3 or a high dose of 10^6 of S. epidermidis (Se) at day+2, +5 and +7 post treatment. Sterile controls were inoculated by vehicule.

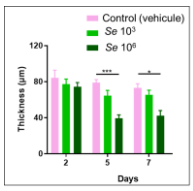

Figure 4: Thickness measurements of living layers of epidermidis. Measurements were conducted on each sample and averaged per group of treatment.

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.

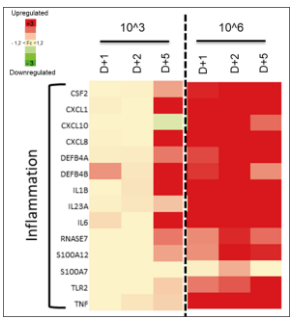

Figure 5: Heatmap representing the expression of inflammation markers after S. epidermidis colonization with 10^3 or 10^6 CFU/cm² compared to the sterile control. Foldchange significatively upregulated are indicated in red whereas those downregulated are in green.

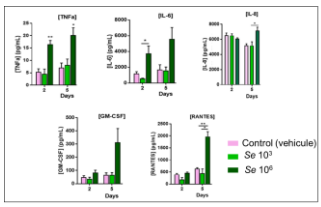

Figure 6: Quantification of TNFα, IL-6, IL-8, GM-CSF (CSF2), and RANTES in the culture conditioned media of sterile controls or LabSkin tissues inoculated with S. epidermidis at a low dose of 10^3 CFU/cm² or a high dose of 10^6 CFU/cm². Quantifications were conducted on individual samples and averaged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

Epidermis differentiation depends on *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) while the filaggrin protein expression is comparable to the control for the tissues inoculated with 10^3 CFU/cm².

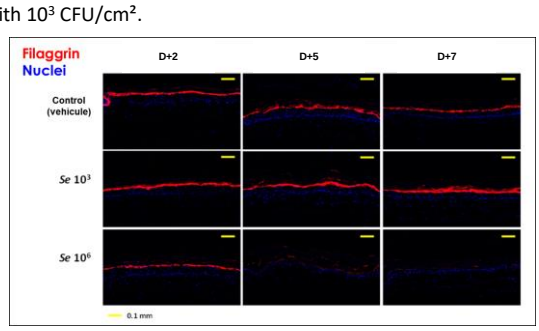

Figure 7: Immunofluorescence staining of the filaggrin protein (red) and nuclei (blue) for LabSkin tissues inoculated with a low dose of 10^3 or a high dose of 10^6 of S. epidermidis (Se) at day+2, +5 and +7 post treatment. Sterile controls were inoculated by vehicule.

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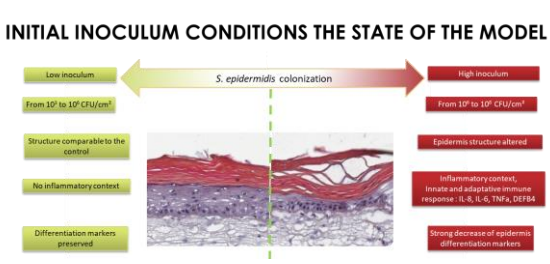

This study shows a first **link between *S. epidermidis* abundance and epidermis structure, differentiation and inflammation context**. All together, these data allow us to develop a simple healthy colonized human skin model. Further *in vitro* studies will be needed including other microbial species 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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Aknowlegments :
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