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In vitro studies of skin microbiota-host interactions based on 3D skin models

Poster 536

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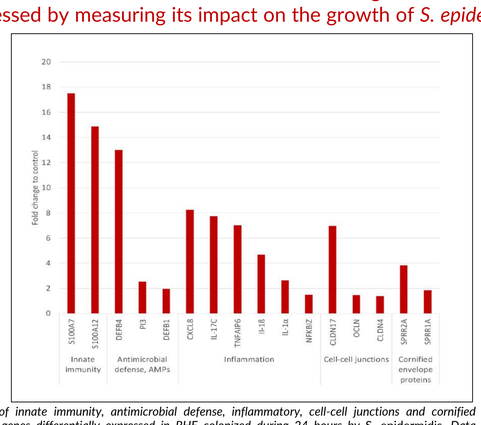
The human skin is host to trillions of microorganisms including bacteria, viruses, and fungi. This natural flora which lives on our skin is called the skin microbiome. Hence, new skincare products are now evolving to better preserve or enhance this natural ecosystem and suitable experimental models are then required for research on the skin microbiome. Currently, Human Reconstructed Epidermis (RHE) models are widely accepted as a valuable tool in dermatological research. On its MicroBIOS Platform, StratiCELL combines skin microbiota key components and 3D RHE, to study the efficacy of dermo-cosmetic ingredients on microbial homeostasis and disorders linked with dysbiosis. StratiCELL is studying commensal and opportunistic strains of the skin including *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Cutibacterium acnes* and *Malassezia furfur*. The RHEs are colonized with these microbiological strains and a two-tiered approach is developed to objectivate the influence of dermo-cosmetic actives. The first is the monitoring of the microorganism's adhesion and growth on the stratum corneum. The second is the study of the epidermal response to bacteria, yeasts and fungi. For each bacterial or fungal species selected to infect RHE, a systematic methodology was applied. According to their characteristics and their metabolism, the protocols of culture, infection, harvesting and counting of CFU (Colony Forming Units) were optimized. The tissue response to infection was studied after a period of growth of the microorganism on the top of the RHE with a transcriptomic tool based on the TaqMan Low Density Array (TLDA) technology. StratiCELL develops a proprietary TLDA that we have named "Skin Response to Microorganisms" to evaluate the expression of 93 key genes selected from full-transcriptome analysis and involved in the skin response to micro-organisms. We selected these 93 genes for their key role in three processes related to bacterial infection, namely inflammation, innate immunity response, and skin barrier structure and homeostasis. The relevance and reliability of these infection biomarkers was then validated by qPCR. For some of them, a quantification by ELISA was performed to confirm that the changes in mRNA expression also affect protein abundance.

Staphylococcus epidermidis

Microbiome-Friendly skin care products are intended to preserve the natural flora of the skin, including the commensal *Staphylococcus epidermidis*. The safeness of skin care product can be assessed by measuring its impact on the growth of *S. epidermidis* in the real context of an *in vitro* 3D RHE, close to human skin.

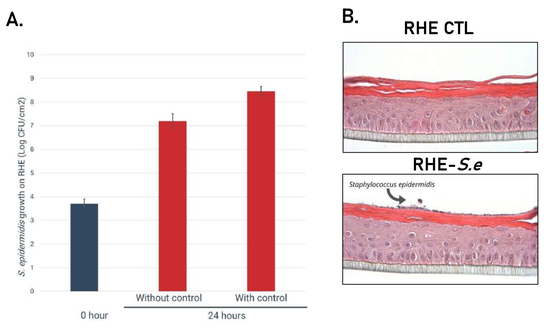
RHE response to S. epidermidis

Allocation of the 93 transcripts selected by StratiCELL to be amplified in a microplate-based quantitative PCR system known as "TaqMan Low Density Array" (TLDA) and referred as "TLDA Skin Response to Microorganisms".



Analysis of innate immunity, antimicrobial defense, inflammatory, cell-cell junctions and cornified envelope genes differentially expressed in RHE colonized during 24 hours by S. epidermidis. *Data extracted form a 93 genes expression analysis using a microplate-based quantitative PCR system known as "TaqMan Low Density Array" (TLDA) carefully designed by StratiCELL and referred as "TLDA Skin Response to Microorganisms". Change in gene expression are expressed as fold change to uncolonized RHE control.*

Colonization of RHE with S. epidermidis



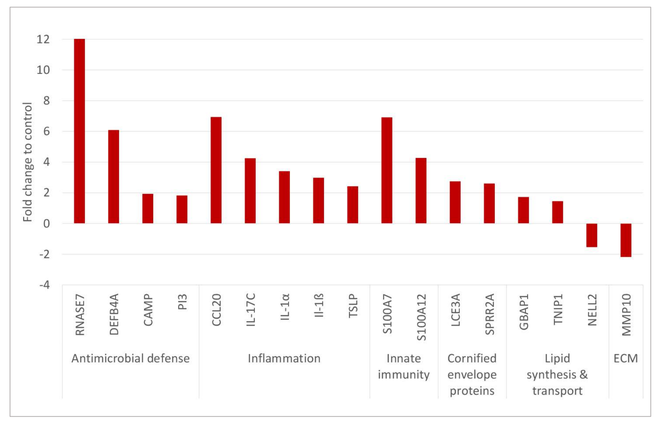
A. *Evaluation of S. epidermidis growth after 24 hours on RHE, in the presence of a growth activator (control) or not, by C.F.U. (Colony Forming Units) counting.* **B.** *Morphological analysis of RHE colonized (RHE-SE) or not (RHE CTL) by S. epidermidis after Hemalun/Eosin staining of paraffin-embedded sections.*

Staphylococcus aureus

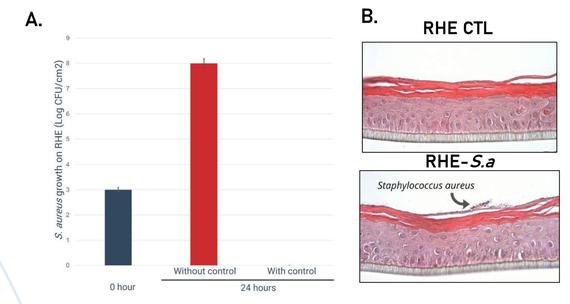
Staphylococcus aureus is a Gram positive bacterium naturally living on the skin. Even though *S. aureus* is harmless in healthy skin, it can invade injured skin and cause serious infection. Understanding the interaction of dermo-cosmetic compounds with *S. aureus* and the skin is crucial to regulate its bacterial activity. This is why StratiCELL has developed a 3D *in vitro* model combining RHE with *S. aureus*.

RHE response to S. aureus

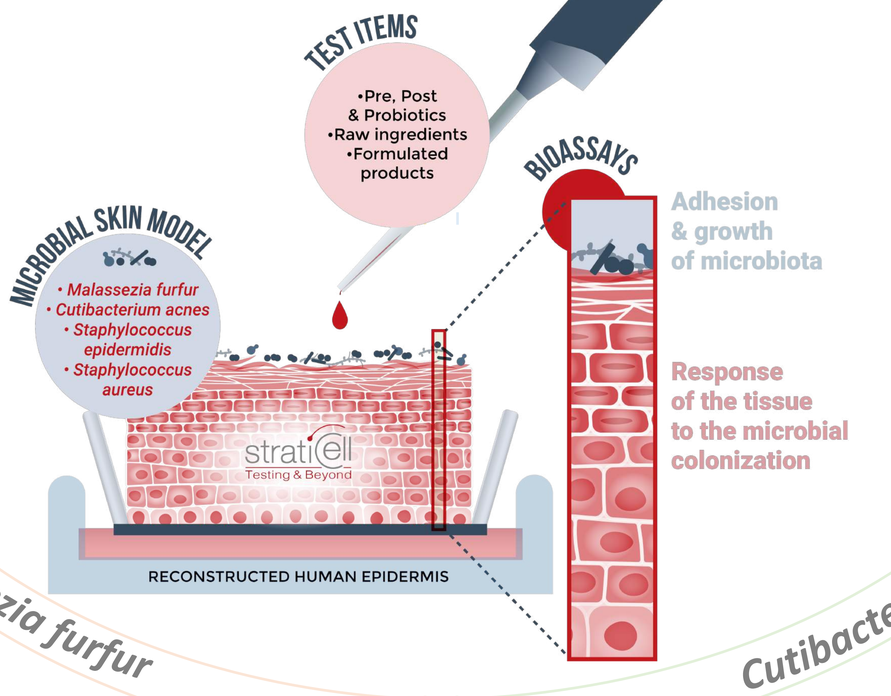
Analysis of antimicrobial defense, inflammatory, innate immunity, cornified envelope proteins, lipid synthesis and transport, and extracellular matrix (ECM) remodeling genes differentially expressed in RHE colonized during 24 hours by S. aureus. *Data extracted form a 93 genes expression analysis using a microplate-based quantitative PCR system known as "TaqMan Low Density Array" (TLDA) carefully designed by StratiCELL and referred as "TLDA Skin Response to Microorganisms". Change in gene expression are expressed as fold change to uncolonized RHE control.*



Colonization of RHE with S. aureus



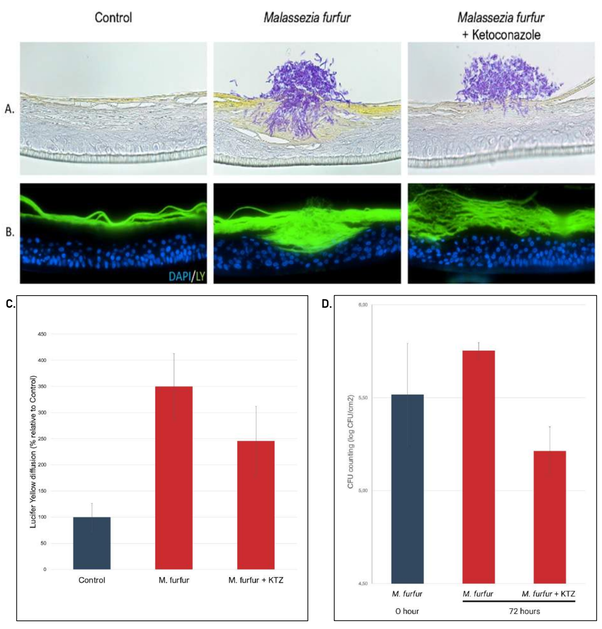
A. *Evaluation of S. aureus growth after 24 hours on RHE, in the presence of a growth activator (control) or not, by C.F.U. (Colony Forming Units) counting.* **B.** *Morphological analysis of RHE colonized by S. aureus (RHE-SA) after Hemalun/Eosin staining of paraffin-embedded sections.*



Malassezia furfur

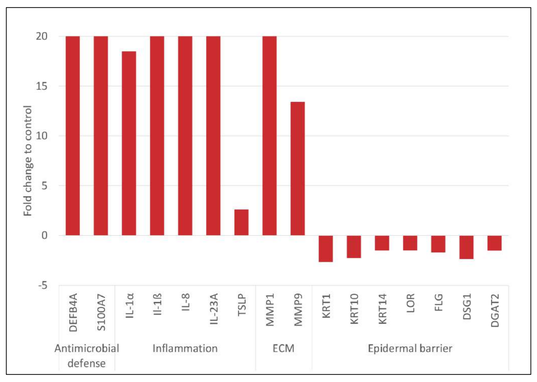
Malassezia furfur is a lipid-dependent yeast naturally living on the skin. However, *M. furfur* overgrowth is associated with skin disorders such as seborrheic dermatitis and dandruff, characterized by a huge spread of yeast invading the epidermis, with detrimental consequences on the skin barrier function. To allow new studies on the efficacy of innovative antifungal actives, StratiCELL has overcame the challenging colonization of RHE by living *M. furfur*. This new model displays a huge reactivity of the tissue, as observed by the expression of key biomarkers.

Colonization of RHE with M. furfur



Representative images of RHE colonized by M. furfur *or not (Control), in the presence of Ketoconazole (KTZ) or not.* **A.** *Periodic Acid Schiff (PAS) staining of paraffin-embedded sections.* **B.** *Lucifer Yellow (LY) fluorescence after out/in epidermal barrier diffusion assay.* **C.** *Quantification of out/in Lucifer Yellow diffusion through the* stratum corneum *of RHE colonized by* M. furfur *or not (Control), in the presence of Ketoconazole (KTZ) or not.* **D.** *Evaluation of* M. furfur *growth after 72 hours on RHE, in the presence of Ketoconazole (+KTZ) or not, by C.F.U. (Colony Forming Units) counting.*

RHE response to M. furfur

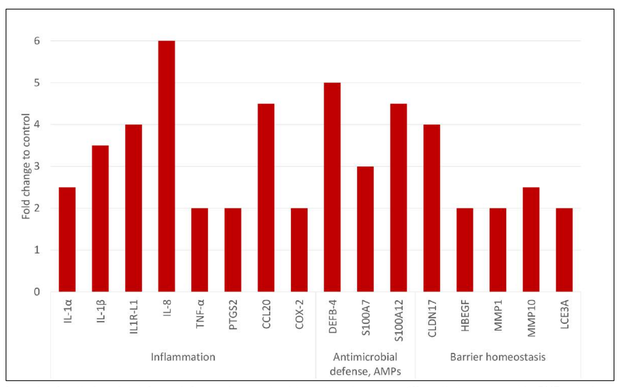


Analysis of antimicrobial defense, inflammatory, extracellular matrix remodeling enzymes (ECM) and epidermal barrier genes differentially expressed in RHE colonized during 72 hours by M. furfur. *Data extracted form a 93 genes expression analysis using a microplate-based quantitative PCR system known as "TaqMan Low Density Array" (TLDA) carefully designed by StratiCELL and referred as "TLDA Skin Response to Microorganisms". Change in gene expression are expressed as fold change to uncolonized RHE control.*

Cutibacterium acnes

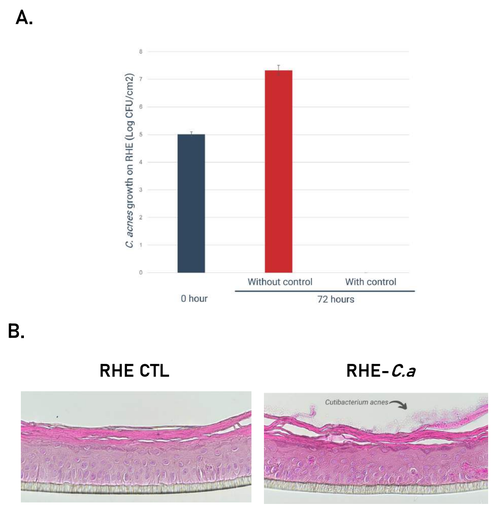
The pathophysiology of acne is related to an increase in the acneic IA1 phylotype of *Cutibacterium acnes*. This anaerobic bacterium of the cutaneous flora feeds on excess of sebum that release short-chain fatty acids responsible for the local inflammatory state and acne spots. Local anti-acne treatments targeting the microflora and/or the production of sebum can reduce and prevent acne spots. In order to study the efficacy of innovative anti-acne treatment, StratiCELL colonizes RHE with a living *C. acnes* strains of IA1 phylotype. Inflammatory response and bacterial adhesion/growth can be studied in this 3D model.

RHE response to C. acnes



Analysis of antimicrobial defense, inflammatory and epidermal barrier genes differentially expressed in RHE colonized during 72 hours by C. acnes. *Data extracted form a 93 genes expression analysis using a microplate-based quantitative PCR system known as "TaqMan Low Density Array" (TLDA) carefully designed by StratiCELL and referred as "TLDA Skin Response to Microorganisms". Change in gene expression are expressed as fold change to uncolonized RHE control.*

Colonization of RHE with C. acnes



A. *Evaluation of* C. ances *growth after 72 hours on RHE, in the presence of a growth activator (control) or not, by C.F.U. (Colony Forming Units) counting.* **B.** *Morphological analysis of RHE colonized by C. acnes (RHE-CA) after Hemalun/Eosin staining of paraffin-embedded sections.*

These four models allow to study both the survival of a microorganism on the surface of the epidermis, and the responses of the skin to the presence of this microorganism. In a context of the evaluation of skin care products to modulate the microbiota and attenuate skin conditions linked to dysbiosis, this double approach enables to investigate the potential effects on the microorganism itself, as well as the response of the tissue to this infection. Those different available *in vitro* 3D models and associated tests offer a promising tool to speed up research, understanding and objectivation of innovative modulators. In conclusion, the infection of *in vitro* reconstituted epidermis by microorganisms constitutes a breakthrough innovation to approach the conditions of *in vivo* infection, allowing among other things the inoculation of strains in a topical way. The challenge is the adaptation of the different models to the specificities and the metabolism of the microorganisms of interest.

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