197. Skin Barrier Disruption Induced by Microbiota Dysbiosis in an Organotypic Skin Model

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Introduction

The skin, the largest organ in humans, serves as a crucial barrier, protecting the body from environmental fluctuations and pathogenic infections. While the majority of microorganisms residing on the skin are harmless or even beneficial, some, like *Staphylococcus aureus*, can become opportunistic pathogens under certain conditions. *S. aureus* is a common commensal found on the skin of 10–20% of healthy individuals but can also colonize lesioned skin in up to 90% of cases of atopic dermatitis (AD), as well as other dermatoses such as psoriasis and acne vulgaris.

This study examines the impact of microbiota dysbiosis on skin health, with a focus on the early biomarkers of barrier disruption linked to *S. aureus* colonization. Dysbiosis, or the imbalance of skin microbiota, is closely associated with dermatological conditions like AD, psoriasis, and chronic wounds. These conditions frequently exhibit symptoms of barrier dysfunction, including increased trans epidermal water loss (TEWL), inflammation, and heightened sensitivity to external irritants. The overgrowth of opportunistic bacteria such as *S. aureus* during dysbiosis can lead to the degradation of key skin structural proteins through the production of proteolytic enzymes, resulting in tissue damage and inflammation.

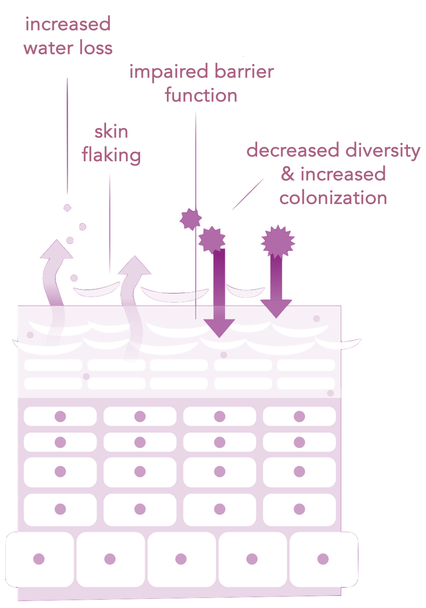

Figure 1A. Skin dysfunctions associated with dysbiosis.

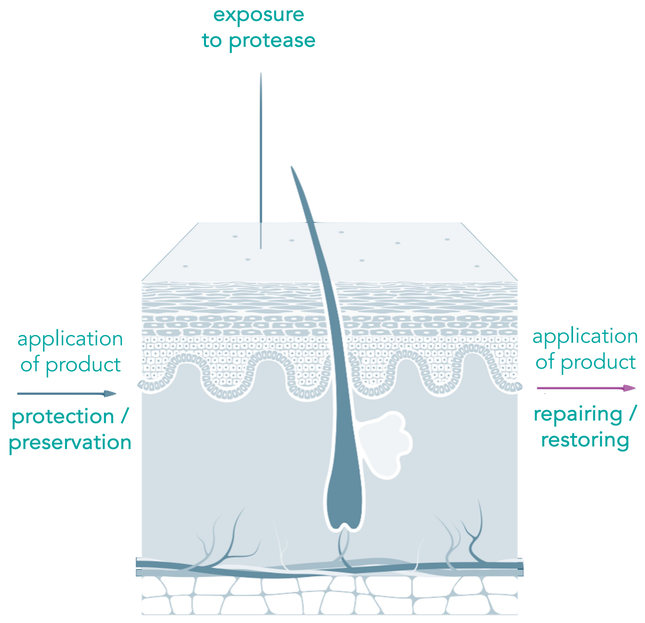

Figure 1B. Experimental Design.

Understanding the initial cellular and molecular effects of dysbiosis-induced barrier disruption is critical for developing effective therapeutic interventions. Advanced skin and scalp models may offer new avenues for restoring microbial balance and preventing the progression of skin disorders by identifying and targeting these early events.

Materials & Methods

Reconstituted human epidermis *in vitro* culture

RHEs were cultured in specific Growth Medium, at 37 °C and humid atmosphere, supplemented with 5% CO_2. Based on these specific effects of opportunistic strains proteases, we assessed the negative effects of dysbiotic microbiome metabolites on skin biomarkers.

Human skin explants *ex vivo* culture

Skin explants were cultured in specific Growth Medium, at 37 °C and humid atmosphere, supplemented with 5% CO_2. Based on these specific effects of opportunistic strains proteases. The negative effects of dysbiotic microbiome metabolites on skin functions or skin biomarkers were assessed.

RHE / skin explants treatments

3D tissues were cultured in specific Growth Medium, at 37 °C and humid atmosphere, supplemented with 5% CO_2 . RHEs and skin edxplants were topically treated for a contact time of 48h, with either the reconstituted protease, or the protease mixed with an active ingredient, or the buffer used for protease dilution for control group. At the end of treatments, the RHEs and the skin explants were sampled and included in OCT for cryopreservation and analyses. Biotics has been sourced from commercial references, as postbiotics' forms (extracts) or as a mix of alive probiotics strains.

Biomarker assessments

After treatments, RHEs and explants cryosections were obtained. Biomarker detection was realised by specific *in situ labeling* with a fluorophore or by immunofluorescence approach. The detection on human keratinocytes was realized by *in situ* labeling. Their fluorescence emission was collected using an epifluorescence microscope. Image analyses and data generation were performed using ImageJ. Data management and statistical analyses were accomplished using GraphPad Software.

In vitro (on skin culture) screening assessments

Human kerainocytes were cultured in specific Growth Medium, at 37 °C and humid atmosphere, supplemented with 5% CO_2 and treated with an active ingredients or product (biotics), then exposed to stressors (either pro-inflammatory cytokines or environmental stress, UV-A). At the end of treatments, the skin cells were fixed and prepared for analyses.

Conclusions

This study highlights the importance of early biomarkers, such as compromised skin barrier integrity, increased inflammatory response, disrupted key components of the skin's cornified layers, and elevated protein carbonylation as indicators of the significant consequences of microbiota dysbiosis. Our findings pave the way for innovative dermatological interventions aimed at preventing, mitigating, or restoring dysbiosis-induced skin damage. These strategies address early causes and prevent late clinical consequences, promoting skin health span.

References

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Results & Discussion

Impaired skin barrier function on skin explants associated to a negative impact on TJs, cell-cell adhesion, skin hydration, oxidative stress & itching.

An *ex vivo* human skin model was developed to replicate the impact of *S. aureus* byproducts (proteases) in the context microbiota dysbiosis. Increased skin permeability was observed upon topical application of *S. aureus* proteases. Skin permeation was associated with the degradation of key structural proteins (**Claudin-1**, involved in cellular tight junctions (TJs); **Desmoglein-1,** involved in cell-cell adhesion, Desmocolin-1, Loricrin (not shown), and **Filaggrin**, implicated in maintaining the skin's hydration thanks to the generation of natural moisturizing factors (NMFs)). In addition, increased levels of inflammatory markers, including **IL-31**, a pro-inflammatory cytokine associated with itching, and S100A8/A9 (not shown), were also observed. Protein carbonylation levels were evaluated as a biomarker of **oxidative damage** and impaired proteostasis.

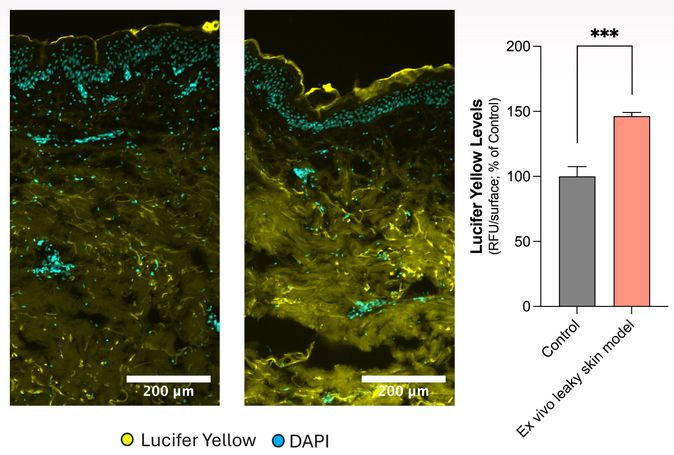
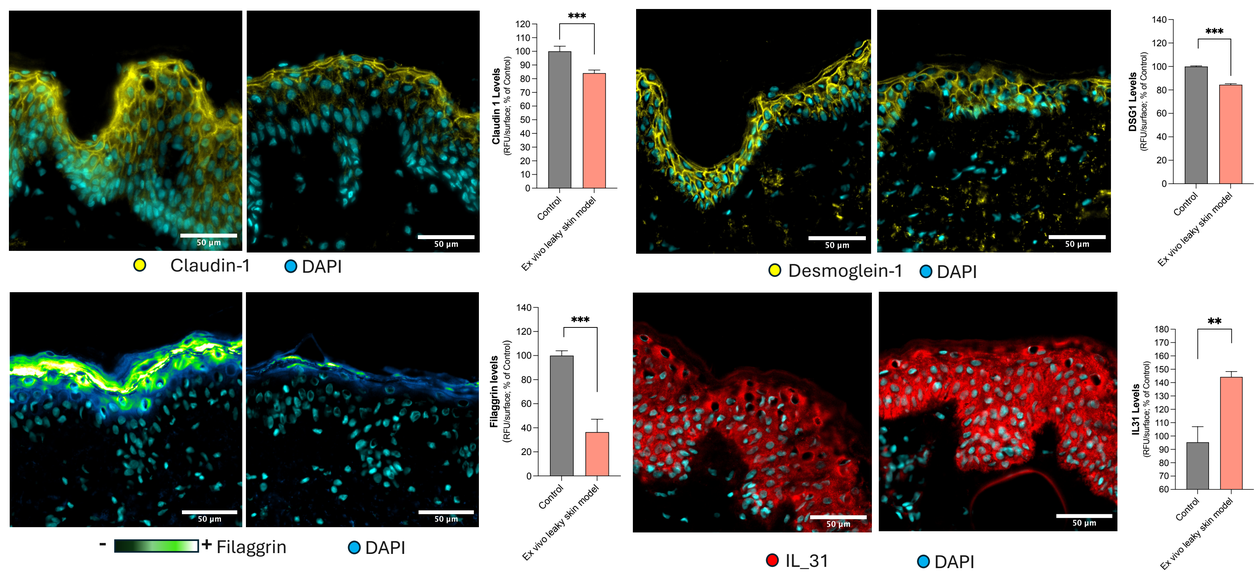


Figure 2. Increased penetration of a fluorescent dye (visualized in yellow) upon contact with proteases.



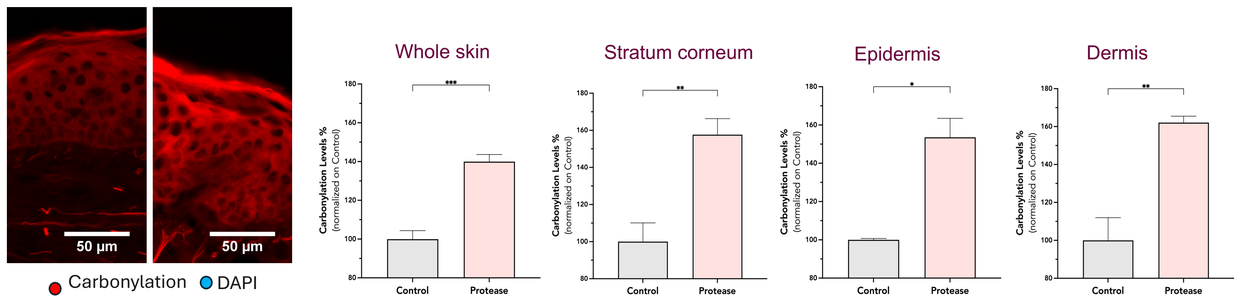


Figure 3. *In situ* (on skin explant sections) visualization of claudin-1 (yellow), desmoglein-1 (yellow), filaggrin (color range), IL-31 (red), carbonylation levels (in red) upon microorganism bioproduct exposure. The quantification of each biomarker is shown as histograms as average values and SD from the mean. Statistics (t-test; *** p<0.001 **p<0.01; *p<0.05).

Biotics preserved the skin from impairment associated to microbiota dysbiosis

Post-biotics derived from commensal skin microorganisms (*B. adolescentis* extract) counteracted protease-induced damage, preserved structural proteins and maintained barrier integrity.

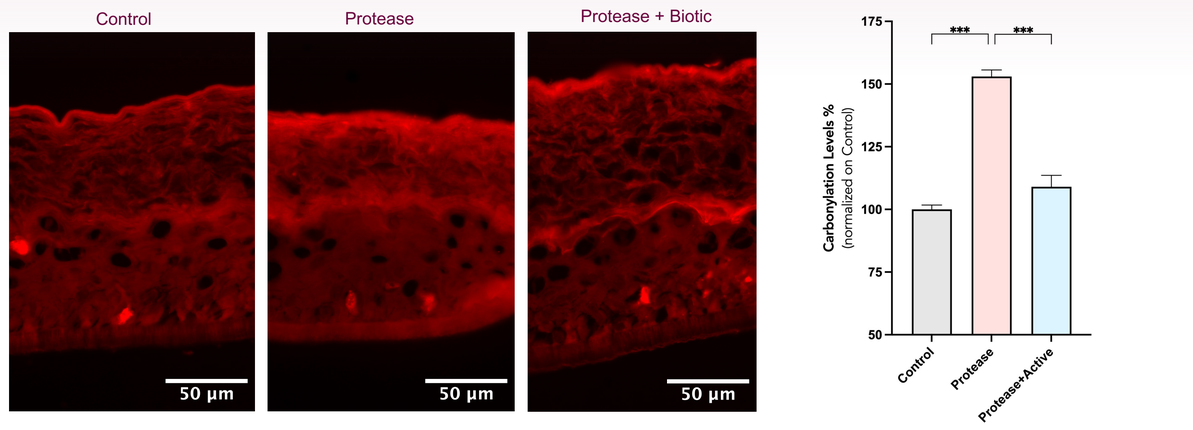


Figure 4: *In situ* (on RHE sections) visualization of carbonylation levels upon treatments (in red). The quantification of carbonylation levels on stratum corneum layers is shown as histograms of average values and SD from the mean. Statistics (ANOVA and Dunnett's post-hoc test versus "Protease"; *** p<0.001).

Postbiotics preserved skin cells from induced oxidative stress (from screening to efficacy)

Probiotics extracts (graph on the left, Figure 5A) and mix of alive probiotics strains: *B. adolescentis* + *L. rhamnosus* (graph on the right, Figure 5B) have been assessed during the screening of top candidate selections and in the preliminary efficacy evaluation using *in vitro* 2D skin models.

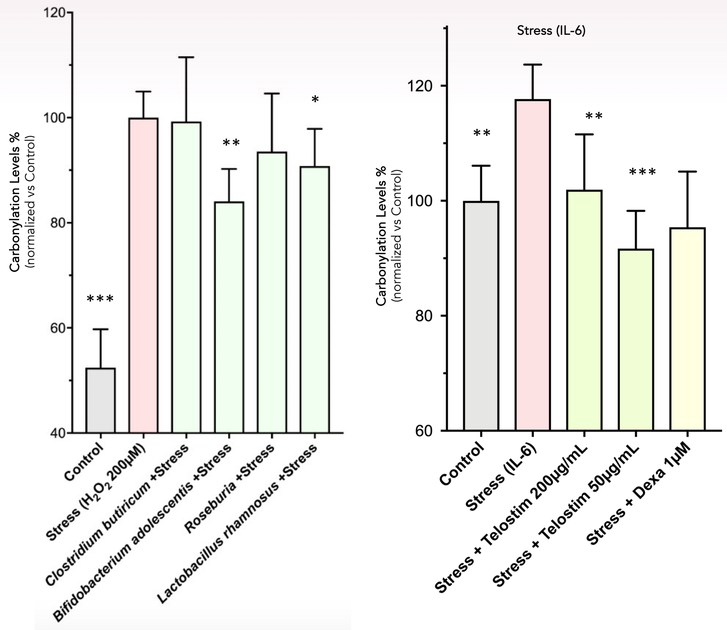


Figure 5 (A; B): Carbonylation levels upon treatments on keratinocytes. The quantification of carbonylation levels on keratinocytes treated (or not) is shown as histograms of average values and SD from the mean. Statistics (ANOVA and Dunnett's post-hoc test versus "Stress"; §p<0.1 *<0.05; **<0.01; *** <0.001).