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"Innovative ex-vivo full skin model for targeting microbiota-induced skin barrier damage"

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1. Introduction

Maintaining a balanced skin microbiota is essential for preserving skin barrier integrity and overall skin health. Dysbiosis of the microbiota—particularly the opportunistic colonization by *Staphylococcus aureus*—plays a pivotal role in skin barrier dysfunction and has been implicated in various inflammatory skin conditions [1, 2].

The skin, the body's largest organ, serves as a vital barrier protecting against environmental fluctuations and pathogenic invasion. This protective function is primarily executed by the epidermis and supported by the host's innate immune responses in conjunction with the resident commensal microbiota. The barrier's integrity can be compromised by numerous factors, including environmental stressors (e.g., UV radiation, temperature, humidity), psychological stress, nutritional status, and systemic physiological conditions [3].

While most microorganisms residing on the skin are harmless or beneficial, some, like *S. aureus*, can become pathogenic under certain conditions. This bacterium, which colonizes approximately 10–20% of healthy individuals asymptomatically, is found on the lesional skin of up to 90% of individuals with atopic dermatitis, as well as those with psoriasis and acne vulgaris [4]. The skin microbiota forms a dynamic ecosystem regulated by processes such as biofilm formation and quorum sensing, which help maintain population balance and host compatibility [5]. However, in dysbiotic states, these regulatory systems can become disrupted, resulting in the proliferation of pathogenic strains, suppression of commensals, and degradation of host tissues due to elevated protease activity.

Analogous to the "leaky gut" syndrome, a dysfunctional skin barrier may exhibit intercellular disruptions in the stratum corneum and deeper layers. This increased permeability allows for

the translocation of microbes, toxins, and allergens, potentially impacting distant organs such as the brain, lungs, and gastrointestinal tract [6].

Despite the broad availability of skin and scalp care products, few specifically address the intracellular damage and tight junction disruption induced by microbiota dysbiosis or pathogenic proteases. Conditions like skin aging, atopic or senile xerosis, and sensitive or dry skin are often associated with dysbiosis and manifest as increased permeability, chronic inflammation, and heightened sensitivity to external aggressors [7].

To advance our understanding of early molecular events in barrier disruption, we developed a novel *ex vivo* human skin model to investigate the effects of *S. aureus* secreted virulence factors, particularly proteases. Our findings support the development of targeted dermocosmetic interventions aimed at preventing, mitigating, or reversing barrier damage by addressing root microbial and molecular causes. By integrating a biomarker-driven approach into efficacy testing, this strategy provides robust scientific evidence to support claims of skin barrier repair and resilience, offering cosmetic brands tools for developing next-generation products that promote skin healthspan (longevity) and protection against environmental and microbiome-related insults.

2. Materials and Methods

Human skin explants or Reconstituted human epidermis culture. Human skin organotypic equivalents (skin explants) or RHEs were cultured in specific Growth Medium, at 37 °C and humid atmosphere, supplemented with 5% CO2. Based on these specific effects of opportunistic strains proteases, we assessed the negative effects of dysbiotic microbiome metabolites on common structural motifs of scalp & skin epidermal tight-junctions.

Reconstituted human epidermis treatment. 3D human skin models were topically treated for a contact time of 48h, with either the reconstituted protease (0.01µg/mL), or the protease mixed with an biotics extract (at 10%), or the buffer used for protease dilution for control group. At the end of treatments, the biological material was sampled and included in OCT for cryopreservation and analyses.

In situ biomarker analyses or Carbonylated proteins assessment. After treatments, cryosections of human explants or RHE were obtained. Carbonylated proteins were labeled *in situ* with a specific fluorophore as described previously [8]. For biomarker analysis, an immunofluorescence approach targeting specific markers was employed. Specific fluorescence signals were detected using an epifluorescence microscope. Image itegration and analyses were performed using ImageJ. Data management and statistical analyses were accomplished using GraphPad Software.

3. Results

Our *ex vivo* model revealed that topical application of *Staphylococcus aureus* proteases significantly compromised skin barrier integrity. This was evidenced by an increase in transepidermal permeability (Figure 1), consistent with the degradation of key structural proteins critical to skin cohesion and barrier function.

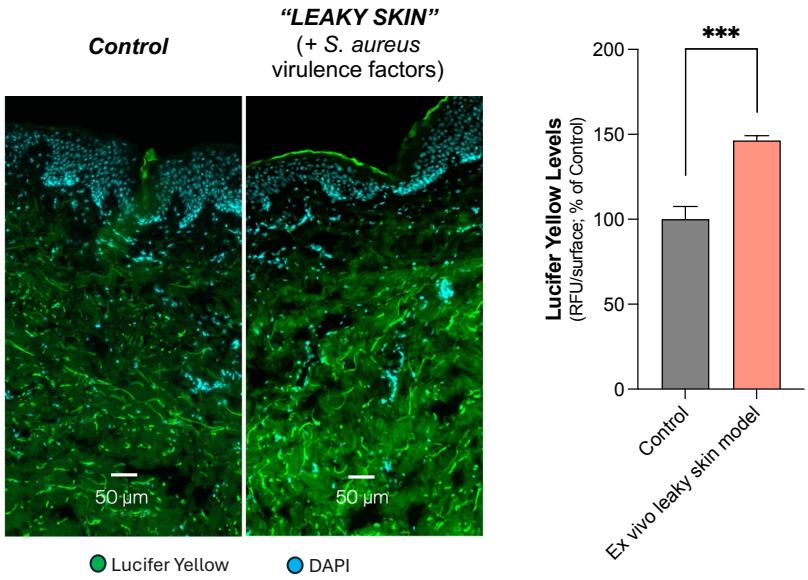

Figure 1. Increased penetration of a fluorescent dye (visualized in green) upon contact with *S. aureus* virulence factors, indicating functional impairment of skin barrier integrity and permeability after treatment. The quantification of fluorophore penetration is shown as histograms as average values and SD from the mean. Statistics (t-test; *** $p<0.001$).

Specifically, immunofluorescence analyses revealed a marked reduction in the levels of **claudin-1, desmoglein-1** (Figure 2), desmocolin-1, loricrin (data not shown), and **filaggrin** (Figure 2), all of which are essential components of tight junctions, desmosomes, and the cornified envelope.

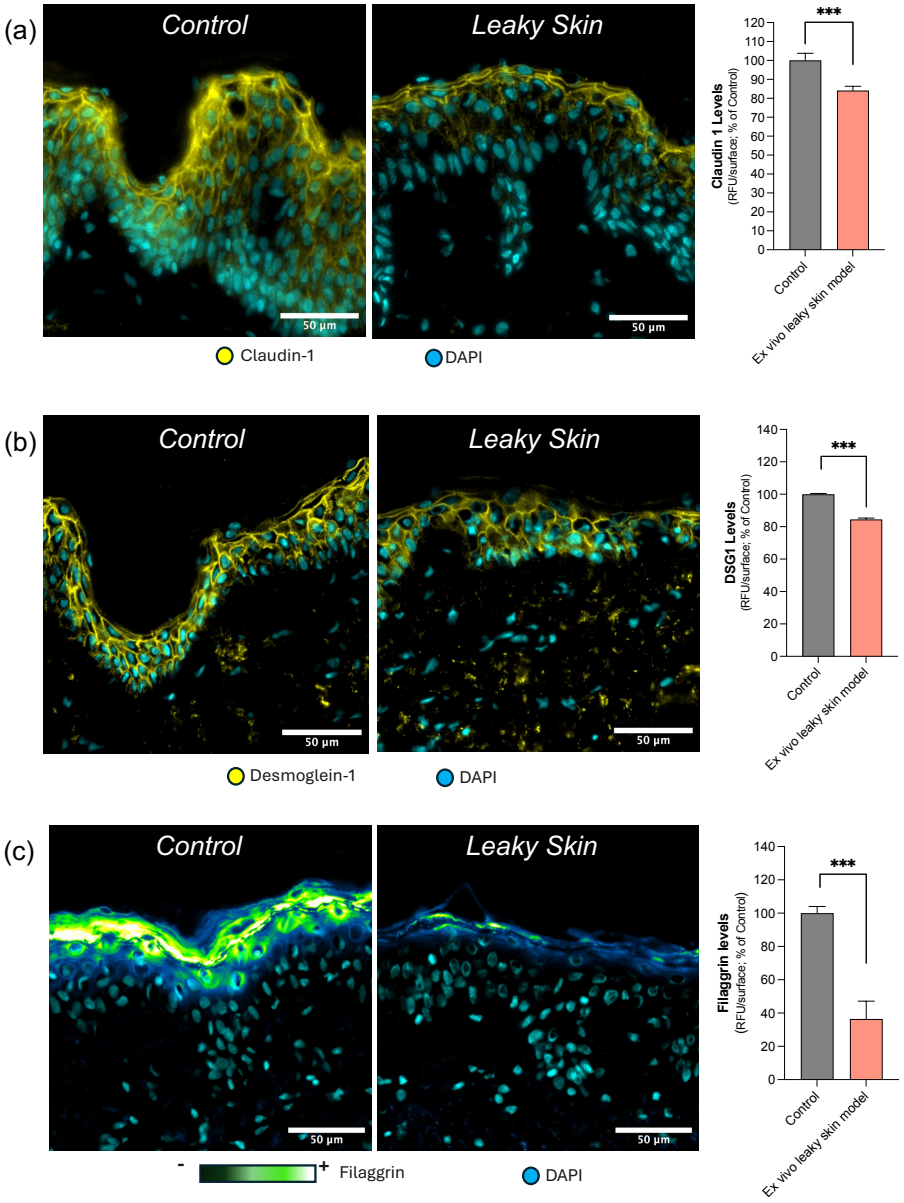

Figure 2. *In situ* (on skin explant sections) visualization of claudin-1 (yellow, 2a), desmoglein-1 (yellow, 2b), filaggrin (color range, 2c), upon microorganism virulence factors exposure. Nuclear detection is shown in in cyan (DAPI). The quantification of each biomarker is shown as histograms as average values and SD from the mean. Statistics (t-test; *** $p<0.001$).

In parallel, we observed a notable upregulation of inflammatory biomarkers. **Interleukin-31** (**IL-31**)—a cytokine associated with pruritus and chronic inflammation—and also the alarmins S100A8/A9 (data not shown) were significantly increased in treated samples, indicating an exacerbated local immune response and activation of innate immune signaling pathways (Figure 3). To further assess cellular stress, we assessed oxidative tissular damage **via *in situ* carbonylation** detection, visualisation and quantification (on the whole skin or *per* anatomical compartment, stratum corneum, epidermis, dermis). Fluorescent labeling of carbonylated proteins showed a significant increase following protease treatment, suggesting that oxidative stress and impaired proteostasis play a central role in the observed tissue disruption (Figure 3).

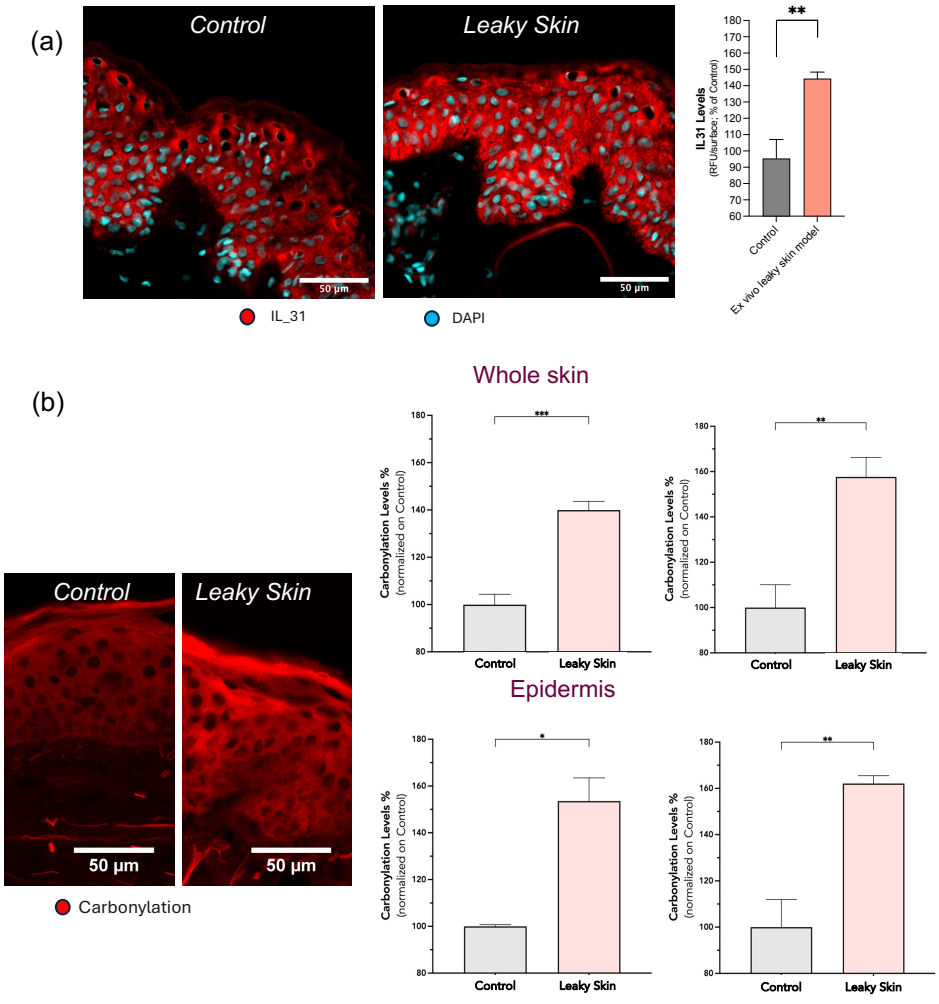

Figure 3. *In situ* (on skin explant sections) visualization of IL-31 (red, upper panels, 3a; nuclear detection in cyan, DAPI) and carbonylation levels (bottom panels, 3b), upon microorganism virulence factors exposure. The quantification of each biomarker is shown as histograms as average values and SD from the mean. Statistics (t-test; *** p<0.001, ** p<0.01). Carbonylation levels were evaluated in the overall skin section or by individual anatomical compartment (stratum corneum, epidermis, dermis) indicating the deeper impact of microbial induced barrier dysfunction.

Importantly, co-treatment with biotics derived from commensal skin microorganisms (e.g., *B. adolescentis* extract) effectively mitigated these deleterious effects 5figure 4). Post-biotic application counteracted protease-induced damage, preserved structural proteins and maintained barrier integrity. These findings demonstrate the potential of *biotics* to counteract dysbiosis-induced skin damage by reinforcing the physical barrier, modulating immune responses, and restoring redox balance.

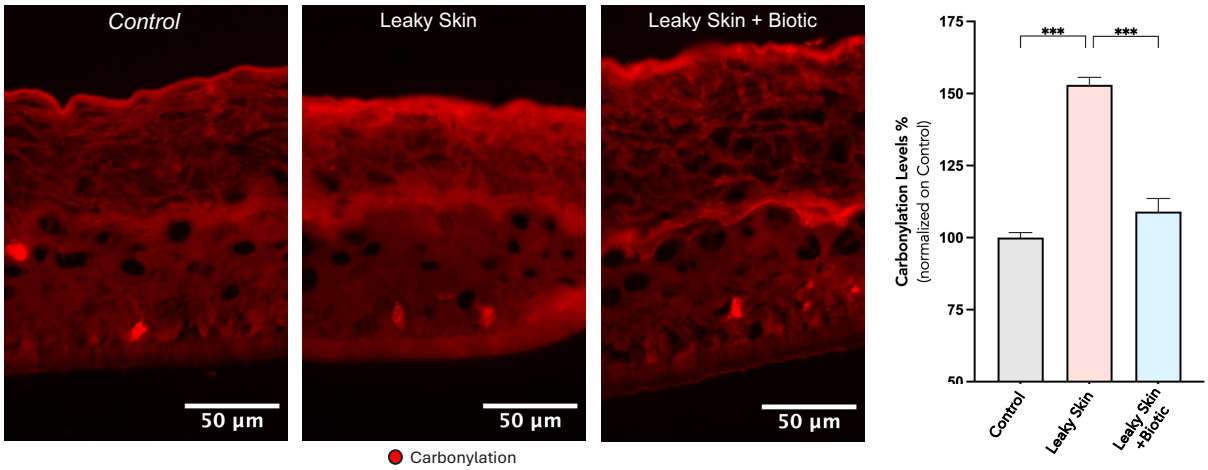

Figure 4. *In situ* (on RHE sections) visualization of carbonylation levels upon treatments (in red). The quantification of carbonylation levels on stratum corneum layers is shown as histograms of average values and SD from the mean. Statistics (ANOVA and Dunnett's post-hoc test versus "Leaky Skin" group;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

4. Discussion

Our findings corroborate previous studies indicating that *S. aureus* proteases play a central role in skin barrier dysfunction by degrading structural proteins and triggering inflammatory responses [2, 4]. Consistent with the "leaky barrier" hypothesis observed in both skin and gut models, our results highlight the importance of tight junction and cornified envelope integrity in preventing dysbiosis-associated damage. Notably, the protective effects of commensal-derived post-biotics observed in our model support growing evidence of their immunomodulatory and barrier-preserving or recovery properties [1].

Overall, our model provides a new sensitive and mechanistically relevant platform for identifying early biomarkers of skin barrier disruption and evaluating therapeutic strategies aimed at microbiota-related skin dysfunction.

5. Conclusion

Our findings support the development of targeted dermocosmetic solutions designed to prevent, mitigate, and reverse skin barrier damage by addressing its root molecular and microbial causes. By incorporating this advanced, biomarker-driven model into efficacy testing, we provide a robust and mechanistically relevant platform for substantiating claims related to

skin barrier protection and repair. This strategy offers cosmetic and therapeutic developers scientifically validated tools to design high-performance products that enhance skin healthspan (longevity) and reinforce resilience against environmental stressors and microbiome-related imbalances. Furthermore, our model show a strong potential for the screening, efficacy evaluation, and optimization of innovative candidates targeting dysbiosis-associated conditions—such as atopic dermatitis, xerosis, and sensitive skin—while also supporting broader applications in preventive and restorative skin health.

References

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