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"A novel ex-vivo scalp model to study the early events associated to microbiota imbalance"

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1. Introduction

The scalp represents a unique and complex microenvironment within the human skin ecosystem. Maintaining a balanced scalp microbiota is essential for preserving the integrity of the stratum corneum, the lipid matrix, and overall scalp health. Dysbiosis of the scalp microbiome—particularly the overrepresentation and opportunistic activity of *Malassezia* species—has been increasingly implicated in barrier dysfunction and scalp-related disorders such as dandruff, seborrheic dermatitis, and general scalp discomfort [1–3].

Malassezia spp. dominate the scalp's fungal flora due to their lipophilic nature and the distinct physiological conditions of the scalp—such as high sebum levels, moisture, specific pH, and unique topography [4,5]. Although typically commensal, these fungi can release extracellular enzymes, including proteases and lipases, which compromise tissue structure and homeostasis under dysbiotic conditions [6,7].

The scalp barrier, similar to cutaneous skin, is crucial for protecting against external aggressors. It comprises structural proteins (e.g., claudins, filaggrin, loricrin), intercellular lipids like ceramides, and tight junctions that collectively regulate permeability and microbial colonization [8,9]. Disruption of these components leads to increased transepidermal water loss (TEWL), inflammation, and altered microbial balance—hallmarks of scalp conditions linked to microbial imbalance [10].

Desmoglein-1 and claudin-1 are essential components of desmosomes and tight junctions, respectively, contributing to intercellular cohesion and barrier integrity. Filaggrin plays a central role in keratinocyte differentiation and hydration of the stratum corneum [11]. Ceramides, key lipids in the extracellular matrix, are critical for maintaining skin hydration and barrier function [10]. Additionally, dysregulation of inflammatory markers such as thymic

stromal lymphopoietin (TSLP) and interleukin-31 (IL-31) has been associated with neuroimmune responses and pruritus in inflammatory skin conditions [12]. Oxidative stress, often marked by protein carbonylation, further exacerbates barrier disruption and inflammation [13].

Despite the widespread use of "*scalp-care*" products, addressing the molecular or microbial origins of barrier dysfunction can represent an innovative and promising approach to managing scalp discomfort associated with microbiota dysbiosis. There remains a pressing need for relevant models that enable both mechanistic understanding and efficacy evaluation of new actives. To meet this need, we developed a novel *ex vivo* human scalp model to evaluate the impact of *Malassezia*-derived proteases on key barrier elements including structural proteins, inflammatory markers, ceramides, and oxidative stress.

Our findings support the development of next-generation, microbiome-conscious scalp solutions aimed at preserving or restoring barrier function and lipid equilibrium for scalp well-being.

2. Materials and Methods

Human Scalp Explant Culture. Human scalp organotypic explants were cultured in specific growth media under controlled conditions (37 °C, 5% CO_2, humidified atmosphere). Explants were topically exposed to *Malassezia*-related proteases (0.01 µg/mL) to simulate dysbiotic insults. The impact on key barrier components, including tight-junction proteins and ceramides, was assessed.

In Situ Biomarker and Carbonylation Analyses. Post-treatment, cryosections were prepared for in situ labeling. Protein carbonylation, as a marker of oxidative damage, was visualized using a specific reactive fluorophore as previously described [13]. Immunofluorescence was employed to detect structural (e.g., claudin-1, filaggrin, desmoglein-1, ceramides) and inflammatory markers (e.g., TSLP, IL-31). Fluorescence imaging was performed with epifluorescence microscopy; analysis was conducted using ImageJ, and statistical evaluation was performed with GraphPad Prism.

3. Results

Scalp Barrier Integrity and Permeability. The exposure to *Malassezia*-derived proteases significantly increased scalp tissue permeability, as shown by enhanced penetration of a dye (Figure 1). This disruption associates with functional impairment of the scalp barrier.

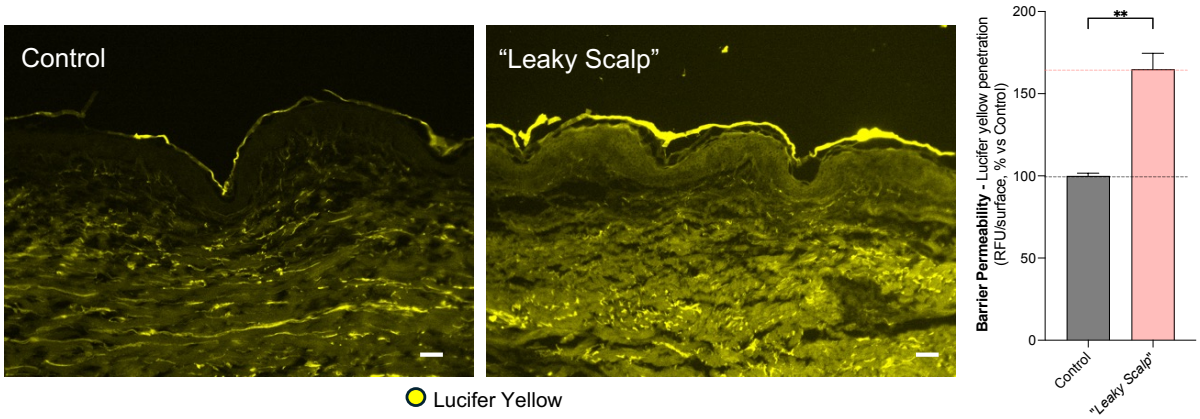

Figure 1. Increased penetration of fluorescent dye (yellow) following *Malassezia* protease exposure, indicating scalp barrier dysfunction. Quantification (mean ± SD) with t-test significance (**$p < 0.01$). Scale bar 100µm.

Structural Protein Degradation.

Immunofluorescence analyses revealed a marked reduction in **claudin-1, desmoglein-1**, **filaggrin**, and loricrin (not shown) *in situ* levels—critical proteins for maintaining scalp integrity (Figure 2). These findings confirm protease-induced structural degradation.

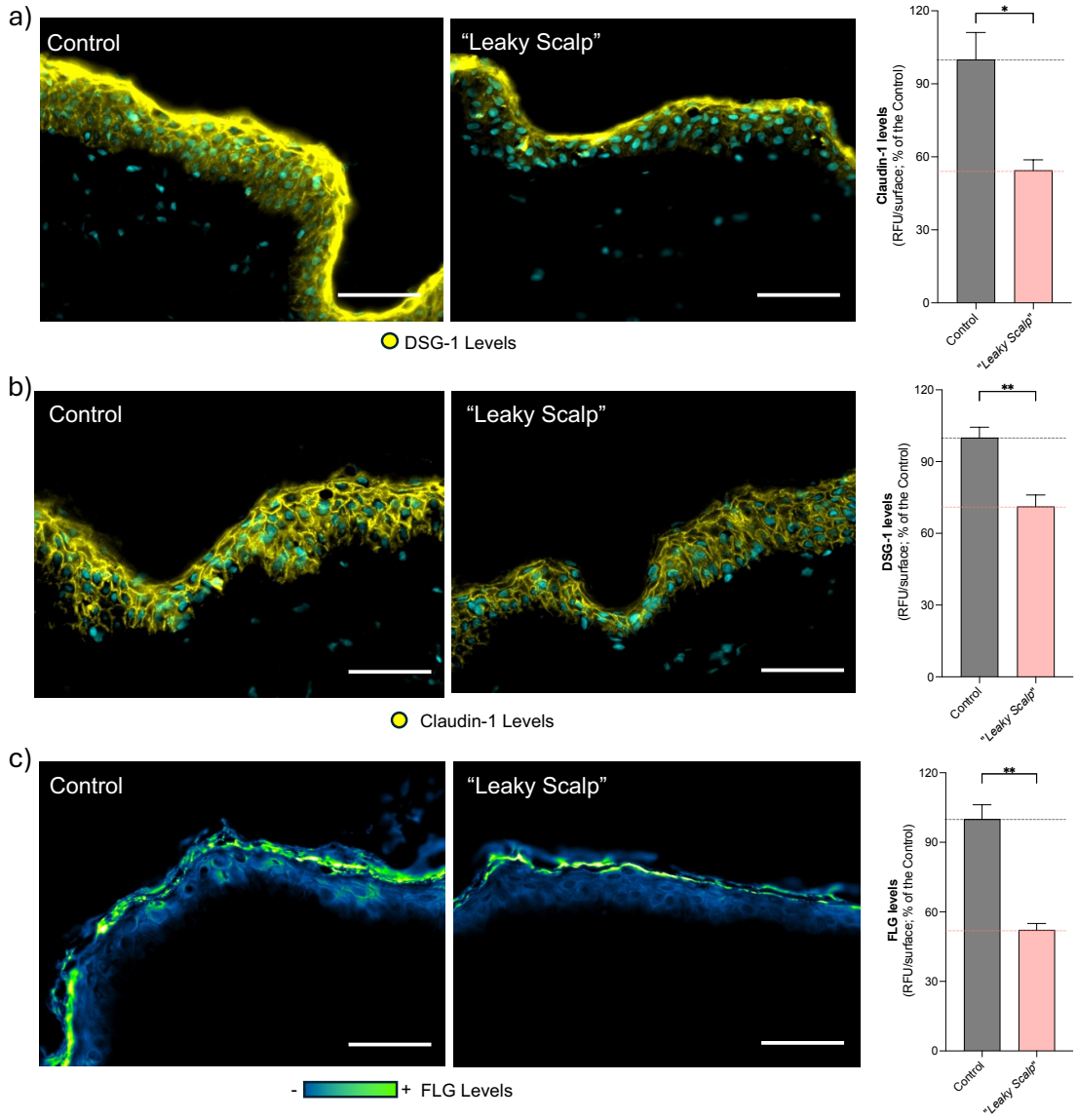

Figure 2. In situ detection of claudin, desmoglein-1, and filaggrin following exposure. Cyan: nuclei (DAPI). Quantification shows significant reduction (**p < 0.01, *p < 0.05) and impairment of these key components of scal biological barrier upon treatment. Scale bar 50µm.

Ceramide Depletion.

Lipid analysis revealed a notable decrease in ceramide levels post-treatment, indicating that *Malassezia* proteases impair not only protein structure but also the **lipidic barrier**, essential for scalp hydration and protection (Figure 3).

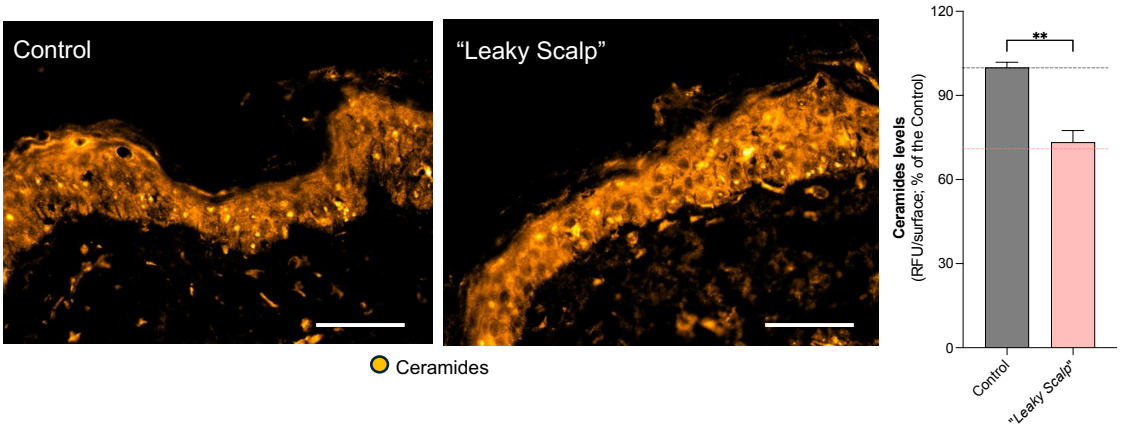

Figure 3. *In situ* (on skin explant sections) visualization of ceramides (in orange). The quantification is shown as histograms as average values and SD from the mean. Statistics (t-test; ** p<0.01). Scale bar 50µm.

Inflammatory and Oxidative Responses.

Increased levels of **TSLP** and **IL-31** were observed (Figure 4), suggesting an inflammatory response typical of scalp irritation. In parallel, **protein carbonylation** levels rose across all skin layers, indicating oxidative stress and compromised proteostasis (Figure 5, next page).

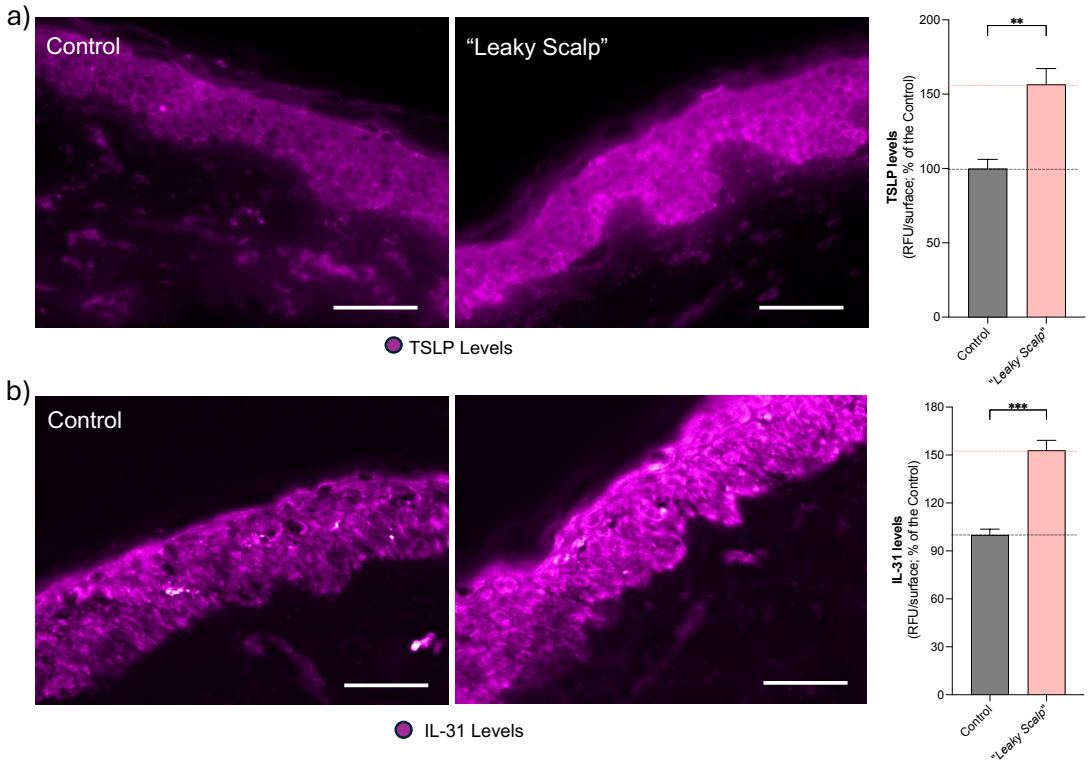

Figure 4. TSLP and IL-31 (4a and 4b, respectively) on scalp interfollicular region. Significant increase in inflammation related markers was detected and visualised (in magenta). Statistics (t- test; *** p<0.001, ** p<0.01). Scale bar 50µm.

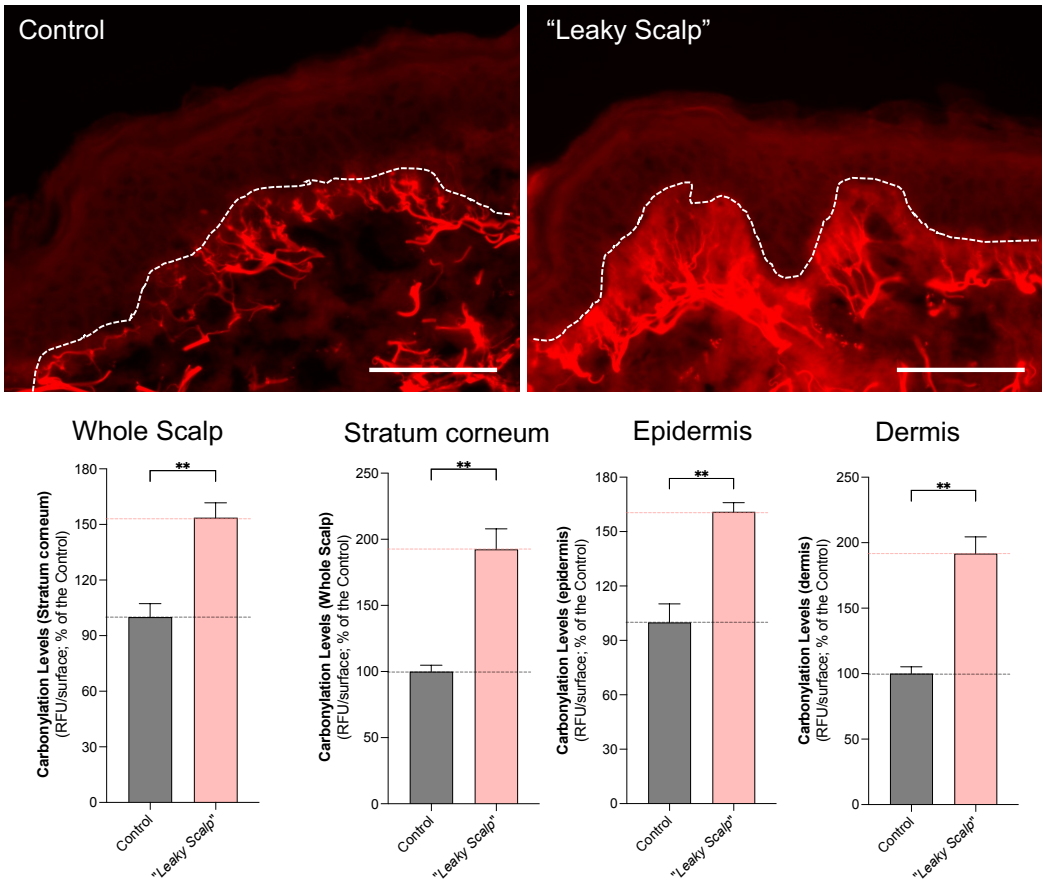

Figure 5. Carbonylation levels across scalp compartments. Significant increase in oxidative damage was detected in both the stratum corneum and deeper layers (visualized in red). Statistics (t-test; **p<0.01). The quantification of carbonylation levels on overall scalp, inter follicular region, or by compartment, is shown as histograms of average values and SD from the mean. White dotted lines separates epidermis and dermis. Scale bar 50µm.

4. Discussion

Our results provide clear evidence that *Malassezia*-derived proteases contribute to scalp barrier impairment by degrading essential structural and lipid components and triggering inflammatory responses [2,4]. The observed reduction in ceramides is especially noteworthy, as these lipids are essential for maintaining scalp hydration and regulating microbial colonization.

The increased carbonylation and IL-31 response parallels findings from gut-skin axis studies, reinforcing the “leaky barrier” hypothesis as a shared pathomechanism. These findings extend previous work on skin models and bring novel insight into scalp-targeted strategies for managing dysbiosis-induced disorders. Importantly, this *ex-vivo* model specifically targeting scalp-microbiota root causes can open the way to new efficacy strategies or interventions to preserve structural integrity and modulate inflammatory and oxidative pathways.

5. Conclusion

This scalp-oriented *ex vivo* model of dysiosis offers a powerful tool for evaluating early molecular and microbial events leading to barrier disruption. It allows mechanistic investigation of both protein and lipid biomarkers, including ceramides, and supports the screening of bioactives or products targeting microbial imbalance. The model revealed the deleterious effects of *Malassezia*-related proteases on scalp integrity.

Our findings could support the development of targeted scalp care solutions aimed at preventing, mitigating, and reversing dysbiosis-driven conditions such as dandruff, seborrheic dermatitis, and general scalp discomfort. This model offers a robust foundation for dermocosmetic innovation focused on restoring **scalp barrier integrity**, lipid balance, and microbiome resilience.

References

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