WHITEPAPER

A Human-Relevant Full-Thickness 3D Skin Model for Atopic Dermatitis

Labskin-AD: Structural, Molecular, and Microbiome Characterisation

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INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease characterised by epidermal barrier dysfunction, immune dysregulation, and epidermal spongiosis (Abdel-Mageed., 2025). Clinically, AD manifests as dry, pruritic, inflamed skin often accompanied by erythema, excoriation, and visible lesions. These symptoms arise from a complex interplay between genetic susceptibility, environmental exposures, immune responses, and alterations in the skin microbiome.

15-20%
of children are affected worldwide

The global prevalence of AD underscores its significant public health burden. AD affects approximately 15–20% of children and 2–10% of adults worldwide (Bylund *et al*., 2020). While many patients experience disease improvement with age, a substantial proportion develop persistent or recurrent disease into adulthood. For these individuals, AD can have a profound impact on quality of life, contributing to sleep disturbance, psychological distress, social stigma, and reduced self-esteem.

2-10%
of adults are affected worldwide

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SKIN BARRIER DYSFUNCTION IN ATOPIC DERMATITIS

The epidermal barrier plays a critical role in maintaining skin hydration, preventing transepidermal water loss, and protecting against environmental insults such as allergens, irritants, and pathogens. In AD, this barrier is structurally and functionally compromised.

Filaggrin (FLG) is a key structural protein essential for epidermal differentiation and barrier integrity. It facilitates keratin filament aggregation and contributes to formation of the cornified envelope and natural moisturising factors. Loss-of-function mutations in the FLG gene are present in approximately 50% of individuals with AD and are strongly associated with increased disease susceptibility and severity (Irvine *et al.*, 2011). Reduced filaggrin expression results in impaired stratum corneum formation, increased permeability, and heightened allergen penetration.

In addition to filaggrin, tight junction proteins such as Claudin-1 are critical regulators of paracellular permeability in the epidermis. Reduced Claudin-1 expression has been reported in AD and is associated with both genetic variation and disease severity (Asad *et al.*, 2016). Disruption of tight junction integrity further exacerbates barrier dysfunction, promoting inflammation and microbial invasion.

IMMUNE DYSREGULATION AND DISEASE PROGRESSION

Barrier impairment in AD initiates and perpetuates immune dysregulation. Antigen penetration and microbial exposure activate keratinocytes and resident immune cells, resulting in the release of pro-inflammatory mediators. In the acute phase of AD, the immune response is dominated by T helper 2 (Th2) cells, which secrete cytokines such as interleukin-4 (IL-4), IL-5, IL-13, and IL-31.

These cytokines drive hallmark features of AD, including:

- B-cell class switching and IgE production
- Eosinophil recruitment and activation
- Suppression of epidermal differentiation and barrier protein expression
- Induction of pruritus and inflammation

Over time, persistent cytokine signalling and barrier disruption contribute to a transition from acute to chronic disease, characterized by epidermal hyperplasia, altered keratinocyte differentiation, and sustained inflammatory signalling.

ROLE OF THE SKIN MICROBIOME

The cutaneous microbiome plays a pivotal role in skin health and immune homeostasis. In AD, barrier disruption alters the microbial landscape, leading to reduced microbial diversity and overgrowth of pathogenic species. *Staphylococcus aureus* is particularly associated with AD severity and disease flares (Park *et al.*, 2013).

Compromised barrier integrity allows *S. aureus* to penetrate deeper into the epidermis, where it outcompetes commensal microorganisms and becomes opportunistically pathogenic (Shi *et al.*, 2018). *S. aureus* produces toxins and superantigens that further amplify immune activation, reinforcing the inflammatory cycle characteristic of AD.

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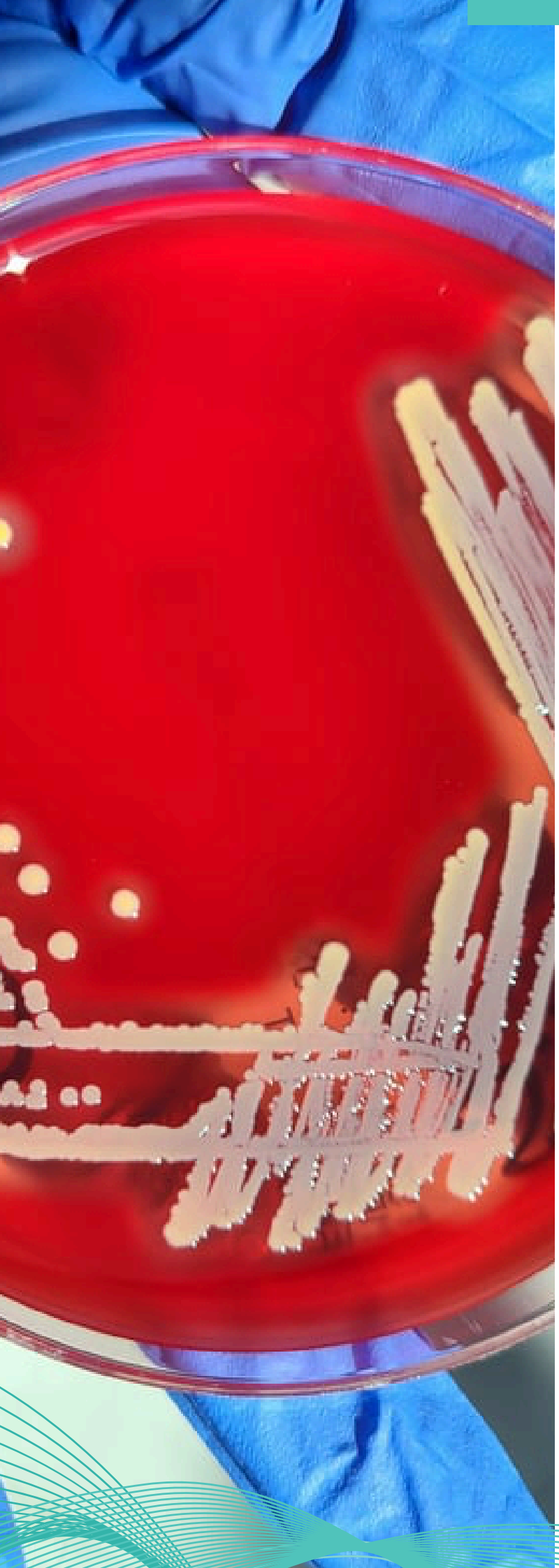

LIMITATIONS OF CURRENT PRECLINICAL MODELS

Despite advances in understanding AD pathogenesis, development of effective therapeutics remains challenging. One major limitation is the lack of physiologically relevant preclinical models. Murine models, while widely used, differ significantly from human skin in structure, immune composition, and barrier properties. Similarly, *ex vivo* human skin explants undergo rapid tissue degradation following excision, limiting their utility for long-term studies and microbiome investigations.

There is therefore a critical need for **living, human-relevant skin models** that faithfully recapitulate the structural, molecular, and microbiological features of AD. Such models would enable mechanistic studies, host–microbiome interaction analysis, and robust therapeutic testing.

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RESULTS AND DISCUSSION

INDUCTION OF AN ATOPIC DERMATITIS–LIKE PHENOTYPE IN LABSKIN

A disease-relevant phenotype was produced using our full-thickness LabSkin-S model exposed to Th2 cytokines IL-4 and IL-13 after reaching full epidermal maturation at day 12 of air–liquid interface (ALI) culture. Cytokines were added into LabSkin maintenance medium for 72 hours to induce inflammation while preserving tissue architecture.

Importantly, cytokine exposure at earlier developmental stages resulted in impaired cell differentiation and an undifferentiated phenotype that failed to resemble either healthy skin or AD pathology. This highlights the importance of temporal control when modelling inflammatory skin diseases *in vitro*.

Following cytokine treatment, LabSkin-AD constructs displayed pronounced morphological changes consistent with lesional AD tissue (**Figure 1**). Compared to standard LabSkin (LabSkin-S), the epidermis exhibited prominent intercellular gaps within the *stratum spinosum* and *stratum granulosum*, indicative of epidermal spongiosis.

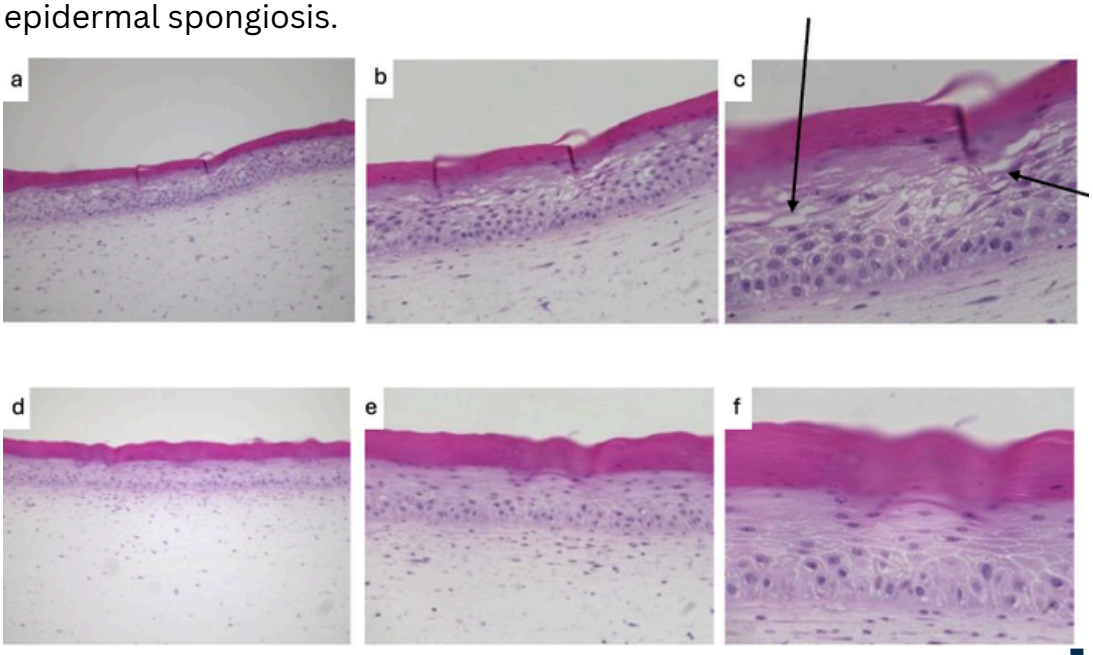

Figure 1. Representative haematoxylin and eosin (H&E)–stained sections of LabSkin-AD, following 72h cytokine incubation, and LabSkin-S models at day 15 of air–liquid interface (ALI) culture. (a–c) LabSkin-AD sections shown at 100x, 200x, and 400x total magnification, respectively. (d–f) LabSkin-S sections shown at 100x, 200x, and 400x total magnification, respectively. Arrows highlight regions in the LabSkin-AD model where skin morphology is altered and cell–cell adhesion appears disrupted, suggesting that key tight junction proteins may be affected.

PERSISTENCE AND REVERSIBILITY OF THE DISEASED PHENOTYPE

Disease progression within LabSkin-AD was monitored over time (**Figure 2**). Intercellular epidermal gaps persisted for up to seven days following cytokine exposure, accompanied by epidermal thinning. These alterations resulted in compromised barrier function, fragmentation, and fraying of the *stratum corneum*.

Following withdrawal of IL-4 and IL-13, the constructs gradually reverted toward a phenotype resembling LabSkin-S. By 7–8 days post-withdrawal, overall epidermal organisation was largely restored. However, residual differences remained, including a thinner, more fragile epidermis and a structurally weakened *stratum corneum* that readily separated from underlying layers. These findings reflect the incomplete barrier recovery often observed clinically in AD patients following resolution of acute inflammation.

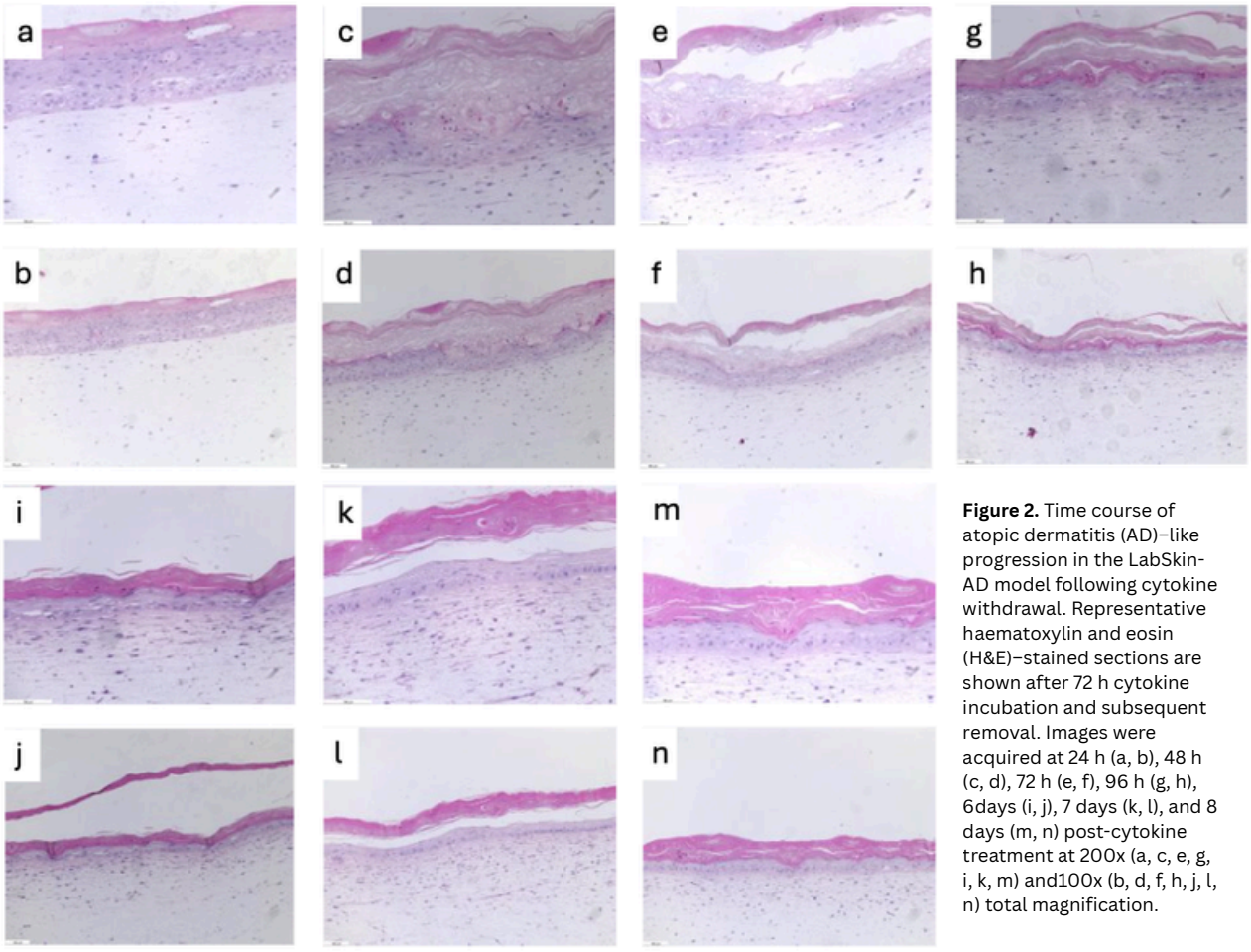

Figure 2. Time course of atopic dermatitis (AD)–like progression in the LabSkin-AD model following cytokine withdrawal. Representative haematoxylin and eosin (H&E)–stained sections are shown after 72 h cytokine incubation and subsequent removal. Images were acquired at 24 h (a, b), 48 h (c, d), 72 h (e, f), 96 h (g, h), 6days (i, j), 7 days (k, l), and 8 days (m, n) post-cytokine treatment at 200x (a, c, e, g, i, k, m) and100x (b, d, f, h, j, l, n) total magnification.

ALTERED EXPRESSION OF BARRIER AND INFLAMMATORY PROTEINS

Immunohistochemical analysis revealed significant alterations in protein expression patterns between human skin, LabSkin-S and LabSkin-AD (**Figure 3**).

Filaggrin staining demonstrated an altered distribution in LabSkin-AD, with fragmented expression, suggestive dysregulated differentiation and abnormal protein processing. Claudin-1 expression was also altered in the epidermal layers.

Notably, Psoriasin (S100A), a well-established marker of cutaneous inflammation, was strongly expressed in LabSkin-AD but absent in LabSkin-S. S100A functions as both an inflammatory mediator and an antimicrobial peptide, and its upregulation is consistent with barrier disruption and immune activation.

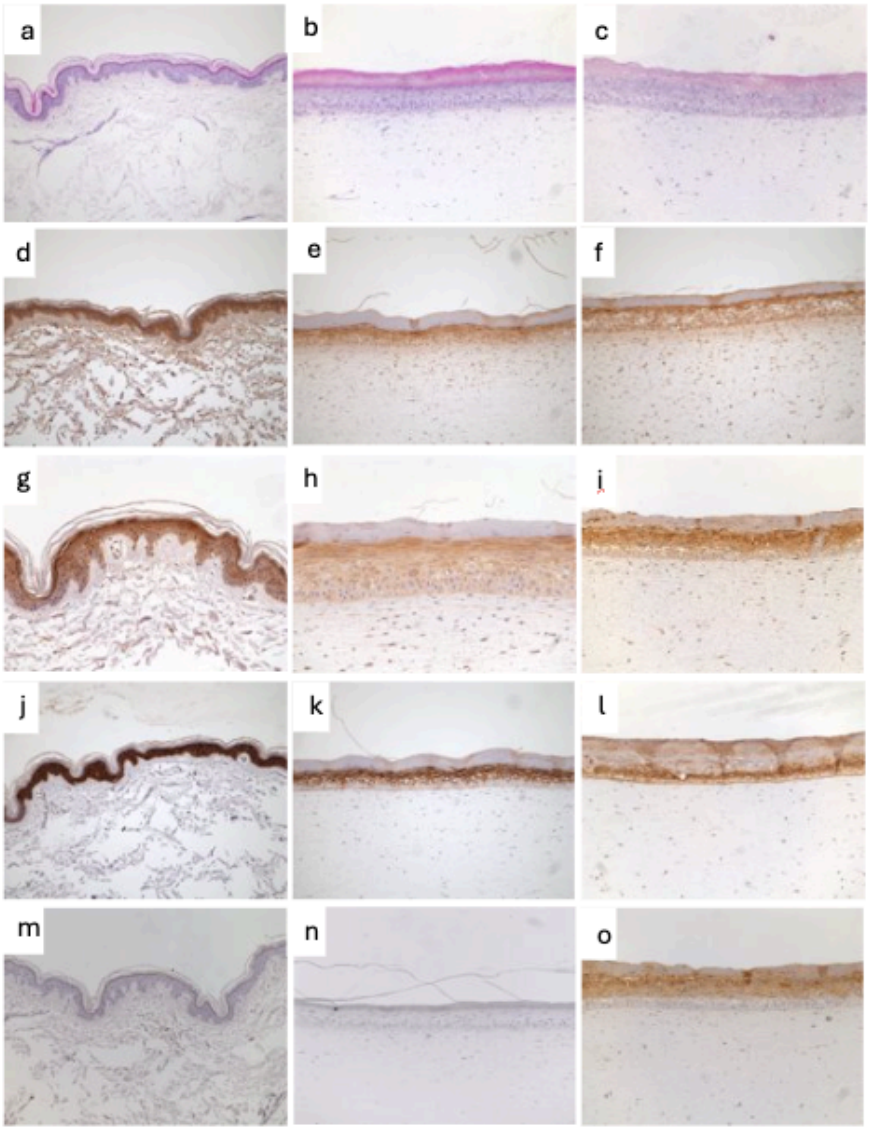

Figure 3. Comparative histological and immunohistochemical analysis of healthy human skin (left column), LabSkin-S (middle column), and LabSkin-AD (right column) models. Representative sections show (a–c) haematoxylin and eosin (H&E) staining; (d–f) desmoglein-1 (DSG1) staining; (g–i) filaggrin staining; (j–l) claudin-1 staining; and (m–o) psoriasin staining respectively. Images taken at 100x total magnification.

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MICROBIAL COLONISATION AND HOST–MICROBIOME INTERACTIONS

A defining advantage of the LabSkin model is its ability to support microbial colonisation. To determine whether this capacity is retained under inflammatory conditions, LabSkin-AD constructs were colonised with either LabSkin's standard three-species (3X mix) microbial consortium (composed of *C. acnes, C. striatum and S. epidermidis)* or with *S. aureus* alone.

Enumeration of viable microorganisms revealed that the three-species consortium (3X mix) established comparable levels on both skin models, with minimal differences in recovery between LabSkin-S and LabSkin-AD. Specifically, Log_{10} CFU cm^{-2} values for *C. acnes, C. striatum, and S. epidermidis* were slightly higher on LabSkin-AD by 0.03, 0.13, and 0.32, respectively; however, these differences were not statistically significant (Welch's t-test, $p>0.05$). This indicates that the presence of cytokine-induced inflammation does not compromise the model's capacity to support a stable, commensal-like microbial community over a 24 h period (**Figure 4a-c**).

In contrast, *S. aureus* colonisation was markedly enhanced on the inflamed model. Bacterial recovery from LabSkin-AD was significantly greater than from LabSkin-S, with a mean increase of 0.86 Log_{10} CFU cm^{-2} ($p = 0.037$). This selective outgrowth of *S. aureus* under inflammatory conditions mirrors the opportunistic expansion characteristic of atopic dermatitis (AD) in clinical settings, thereby validating the LabSkin-AD model as a relevant platform for studying host–pathogen dynamics in diseased skin (**Figure 4d**).

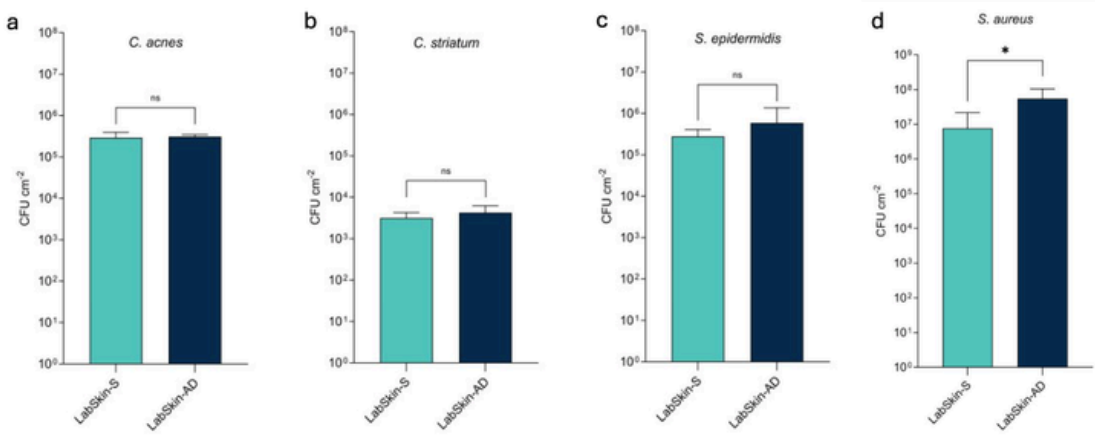

Figure 4. Bacterial recovery from LabSkin models following mono- and poly-bacterial colonisation. Colony-forming units per centimeter squared (CFUcm^{-2}) of (a) *Cutibacterium acnes*, (b) *Corynebacterium striatum*, (c) *Staphylococcus epidermidis*, and (d) *Staphylococcus aureus* recovered 24 h post-incubation. The three-species consortium (3X mix: *S. epidermidis, C. striatum,* and *C. acnes*) was co-cultured on LabSkin-S and LabSkin-AD models (n=5). *S. aureus* was cultured alone on separate, independent constructs (n=5). Data are presented as mean ± SD. NS- Not statistically significant. * -p≤0.05.

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THERAPEUTIC VALIDATION USING A JAK INHIBITOR

To assess the utility of LabSkin-AD for therapeutic testing, the clinically approved Janus kinase (JAK) inhibitor “Upadacitinib” was evaluated. Following the initial 72-hour cytokine exposure, Upadacitinib was added to the culture medium while IL-4 and IL-13 treatment was maintained. After the therapeutic window, constructs were returned to standard maintenance media.

Upadacitinib-treated constructs exhibited a markedly accelerated reversion toward the LabSkin-S phenotype compared to PBS-treated controls (**Figure 5a**). By day 20 ALI (96 hours post-cytokine withdrawal), treated constructs showed substantially improved epidermal architecture, and restored barrier organisation (**Figure 5b**). Structural improvements were already evident by day 19 ALI, underscoring the model’s sensitivity to pharmacological intervention.

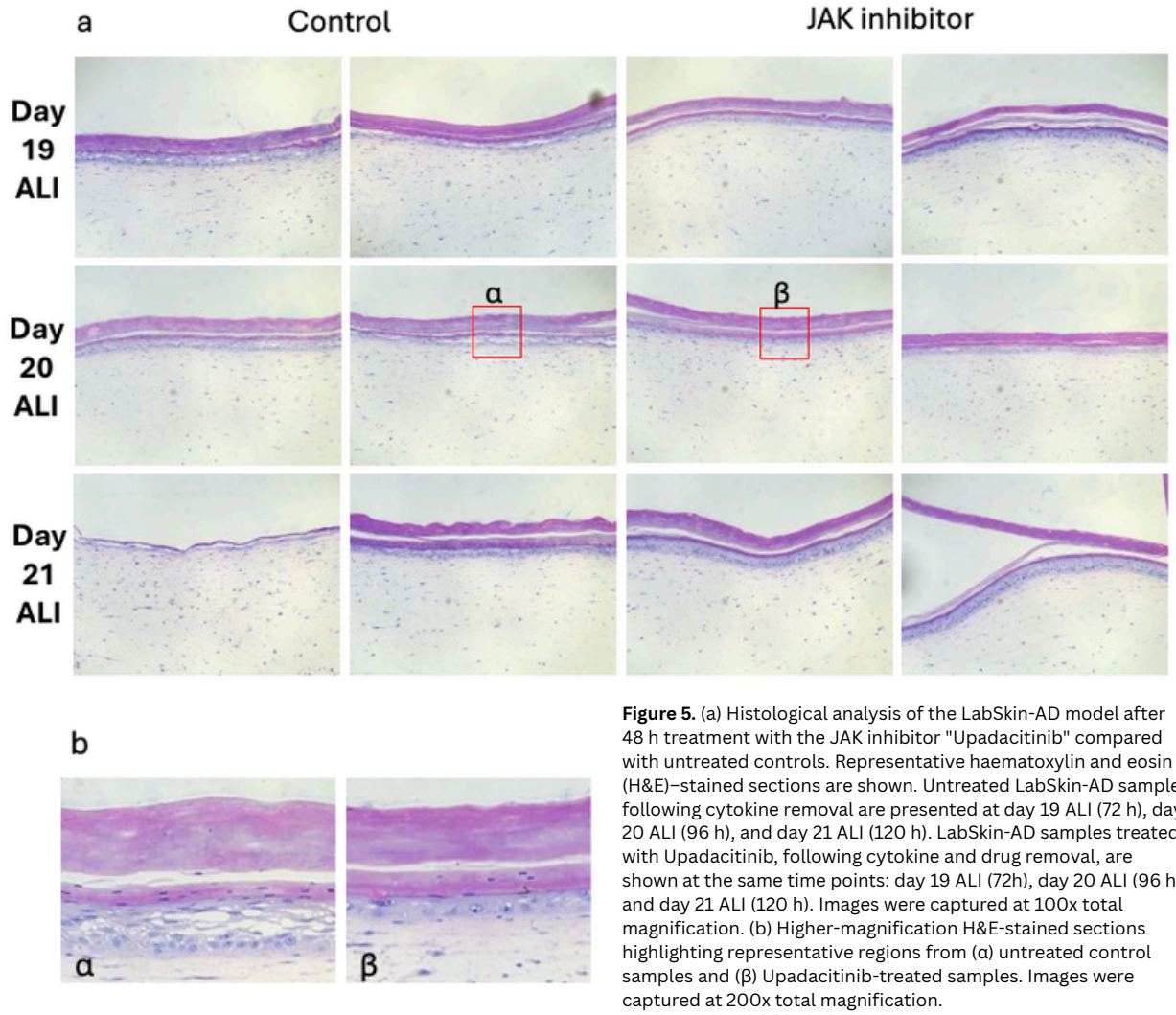

Figure 5. (a) Histological analysis of the LabSkin-AD model after 48 h treatment with the JAK inhibitor "Upadacitinib" compared with untreated controls. Representative haematoxylin and eosin (H&E)–stained sections are shown. Untreated LabSkin-AD samples following cytokine removal are presented at day 19 ALI (72 h), day 20 ALI (96 h), and day 21 ALI (120 h). LabSkin-AD samples treated with Upadacitinib, following cytokine and drug removal, are shown at the same time points: day 19 ALI (72h), day 20 ALI (96 h), and day 21 ALI (120 h). Images were captured at 100x total magnification. (b) Higher-magnification H&E-stained sections highlighting representative regions from (α) untreated control samples and (β) Upadacitinib-treated samples. Images were captured at 200x total magnification.

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PROTEOMIC INSIGHTS INTO DISEASE MECHANISMS

To further elucidate molecular changes underpinning the observed phenotypes, unbiased proteomic analysis was performed (University of Leicester) using mass spectrometry. 6536 proteins were identified in the analysis, with the observed protein profile clearly distinguishing between Labskin-AD and Labskin-S. 1836 proteins were significantly altered in LabSkin-AD relative to LabSkin-S, including proteins relevant to AD pathology (**Figures 6a and 7a**).

Upregulated proteins were predominantly associated with immune activation downstream of IL-4 and IL-13 signalling, including components of the JAK-STAT pathway (**Figure 6b**). Elevated expression of IL-12 and IL-19 was also observed, indicating amplification of immune signalling networks. *In vivo*, such sustained cytokine production promotes immune cell recruitment and perpetuates chronic inflammation.

Interestingly, Claudin-1 expression was significantly elevated relative to LabSkin-S ($p<0.05$). While reduced Claudin-1 expression is linked to AD susceptibility, cytokine-induced upregulation may represent a compensatory response aimed at restoring barrier integrity. This highlights the dynamic and context-dependent regulation of barrier proteins in inflammatory skin disease.

Downregulated proteins included Filaggrin, Involucrin, and Keratin-1 (**Figures 7a i–iii**), all critical markers of late-stage keratinocyte differentiation. Pathway analysis revealed substantial disruption of keratinocyte differentiation and RNA processing pathways, consistent with impaired epidermal maturation and reduced transcriptional activity (**Figure 7b**).

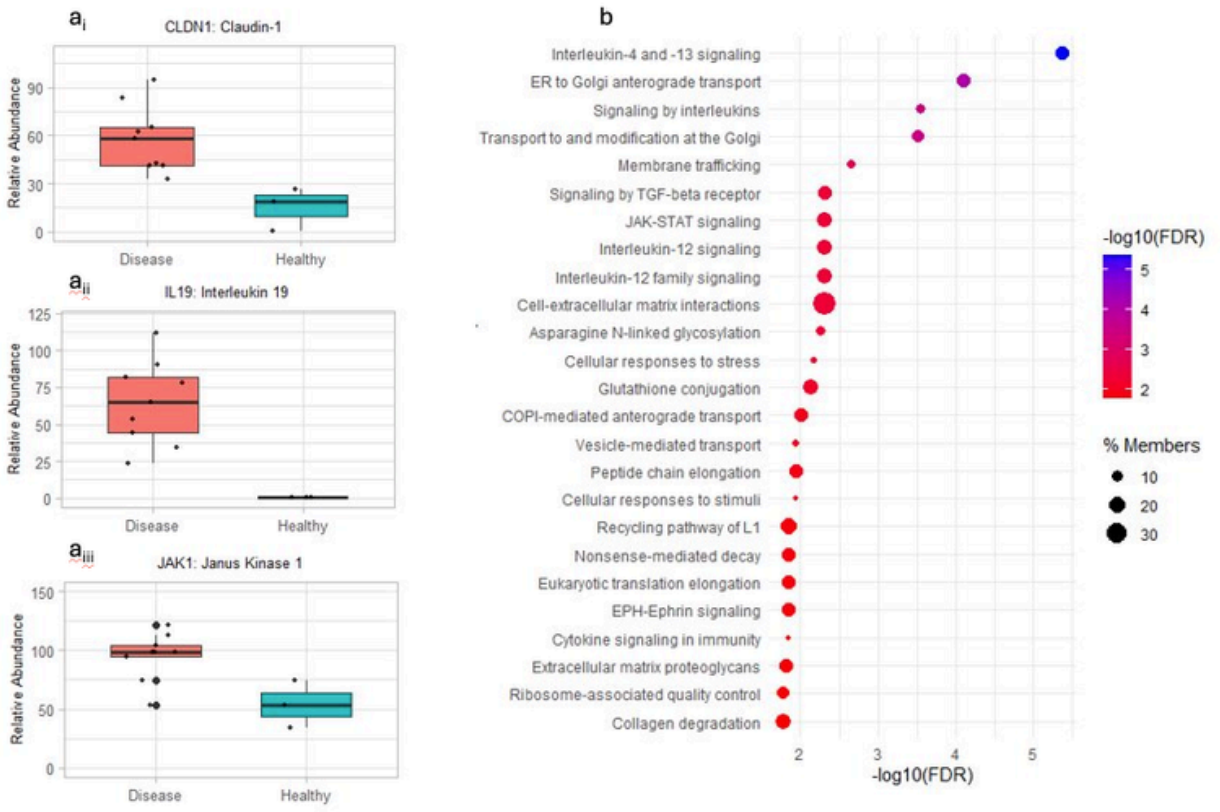

Figure 6. Proteomic analysis highlighting proteins and pathways upregulated in the LabSkin-AD model. (a) Box-and-whisker plots showing the relative abundance of selected proteins in LabSkin-AD (diseased) compared with LabSkin-S (healthy): claudin-1 (ai),interleukin-19 (aii), and JAK1 (aiii). (b) Bubble plot depicting significantly upregulated biological pathways.

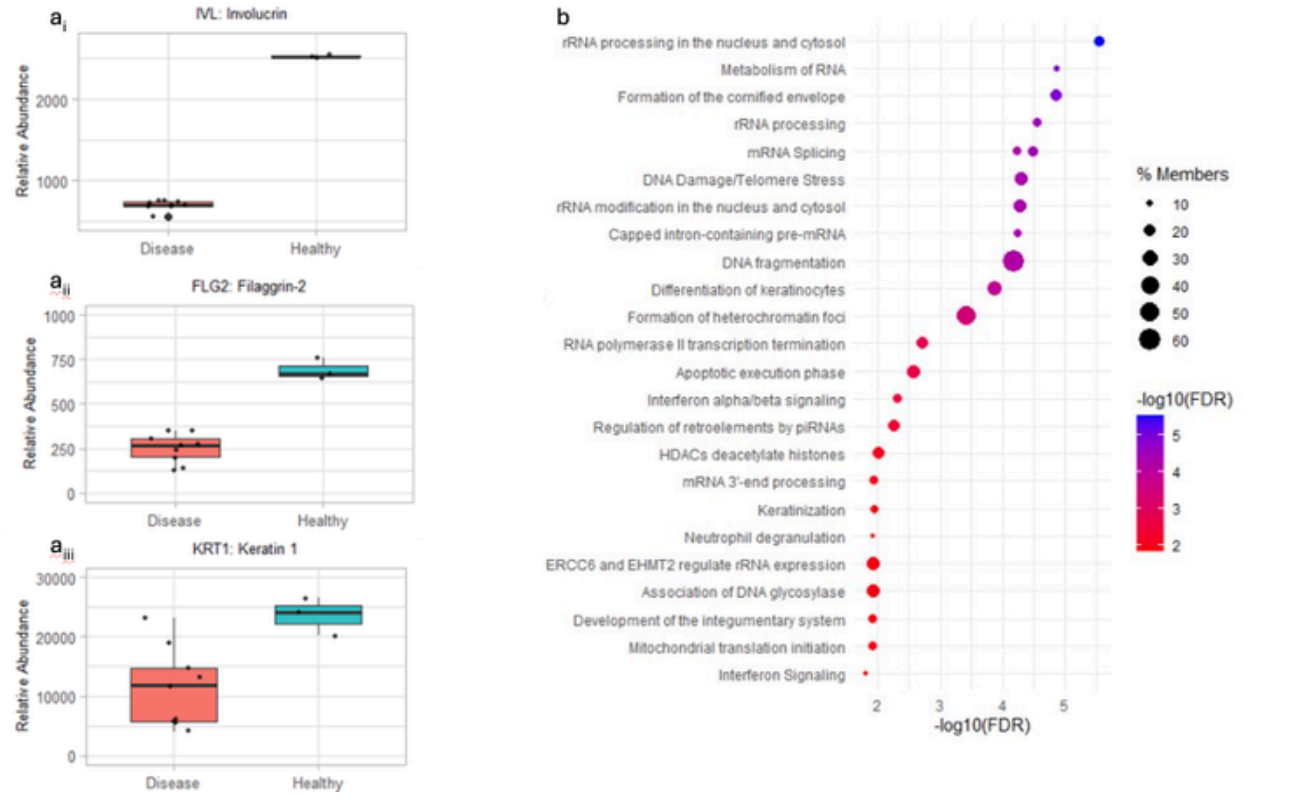

Figure 7. Proteomic analysis highlighting proteins and pathways down regulated in the LabSkin-AD model. (a) Box-and-whisker plots showing the relative abundance of selected proteins in LabSkin-AD (diseased) compared with LabSkin-S (healthy):involucrin (i), filaggrin-2 (ii), and keratin-1 (iii). (b) Bubble plot depicting significantly down regulated biological pathways.

Conclusion

LabSkin-AD represents a robust, physiologically relevant *in vitro* model of atopic dermatitis that captures key structural, molecular, and microbiological hallmarks of the disease. By combining controlled cytokine-induced inflammation with a full-thickness human skin equivalent capable of microbial colonisation, the model bridges an important gap between traditional preclinical systems and human disease.

The model responds appropriately to clinically relevant therapeutics, such as JAK inhibition, and provides mechanistic insights into immune signalling, barrier dysfunction, and host–microbiome interactions. While further refinement - such as incorporation of immune cells or vascular components - will enhance biological complexity, LabSkin-AD already offers a powerful platform for therapeutic screening and disease modelling.

Key Findings and Takeaways

- **Th2 cytokines IL-4 and IL-13 successfully induce an AD-like phenotype** in mature full-thickness LabSkin-S constructs without compromising tissue development.
- **LabSkin-AD recapitulates hallmark features of atopic dermatitis**, including epidermal spongiosis, barrier disruption, altered differentiation, and inflammatory protein expression.
- **Key barrier proteins (Filaggrin, Involucrin, Keratin-1) are significantly downregulated**, aligning with clinical observations in AD patients.
- **Inflammatory markers such as Psoriasin (S100A) are robustly upregulated** in the diseased model.
- **LabSkin-AD supports stable microbial colonisation**, enabling investigation of host–microbiome interactions under inflammatory conditions over 24 h period.
- ***Staphylococcus aureus* exhibits enhanced colonisation in LabSkin-AD**, mirroring its opportunistic expansion in clinical AD.
- **Upadacitinib accelerates tissue recovery and suppresses inflammation**, validating the model for therapeutic testing.
- **Proteomic analysis reveals activation of JAK-STAT and immune signalling pathways**, providing mechanistic insight into disease progression and treatment response.
- **LabSkin-AD offers a human-relevant, reproducible alternative to animal models**, supporting drug discovery and translational research in atopic dermatitis.

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