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Reconstructing Skin Microbiome via a Two-Step AI-Driven Regimen Involving Targeted Microbial Removal and Beneficial Reintroduction

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1. Introduction

The human skin microbiome constitutes a dynamic and complex ecosystem, essential for maintaining cutaneous health and homeostasis. Dysbiosis—characterized by the overgrowth of pathogens such as *Cutibacterium acnes* and *Staphylococcus aureus*—is implicated in chronic inflammatory conditions, including acne, atopic dermatitis, and rosacea. Conventional treatments, such as broad-spectrum antimicrobials or probiotics, often yield limited and inconsistent efficacy. These shortcomings are largely attributed to colonization resistance and biofilm resilience.

To overcome these limitations, we propose a paradigm shift that prioritizes ecological precision and sustainable microbial modulation. Here, we present a two-step, AI-driven regimen designed to selectively eliminate pathogenic microbes and promote the reintroduction of beneficial strains. The strategy leverages antimicrobial peptides (AMPs) identified via our proprietary AI-powered peptide array platform for targeted clearance, followed by recolonization using ammonia-oxidizing bacteria (AOB) such as *Nitrosomonas eutropha*. The platform integrates microbial diagnostics, AMP design, and microbial restoration to form a closed-loop solution for personalized skin microbiome modulation.

2. Materials and Methods

2.1 AI-Guided AMP Discovery

Antimicrobial peptides (AMPs) were identified using a proprietary AI-powered platform that integrates high-density silicon-based peptide arrays and deep learning. The array covers ~3 million pentameric peptides and enables ultra-fast binding analysis via imaging-based detection (>1000× ELISA sensitivity, <3h). AI models trained on binding data predicted novel candidates beyond the array space. Top-ranked peptides were synthesized and structurally validated via mass spectrometry, circular dichroism (CD), and NMR.

2.2 Antimicrobial Activity and Stability

Minimum inhibitory concentrations (MICs) were determined using standard broth microdilution assays against key skin pathogens—*S. aureus*, *C. acnes*, and *S. epidermidis*. Assays were conducted in triplicate to ensure reproducibility. Biofilm disruption was evaluated by crystal violet staining and confocal microscopy. Stability testing was performed under simulated topical stress (sweat, sebum, proteases), confirming AMP retention of activity over 24 months under accelerated storage conditions.

2.3 Biofilm Disruption Assays

AMPs were tested for their ability to disrupt in vitro biofilms of *C. acnes* and *S. aureus*. Biofilm biomass was quantified via crystal violet staining, while bacterial viability was assessed by confocal laser scanning microscopy (CLSM). Structural disruption was visualized using scanning electron microscopy (SEM), and biofilm-associated gene expression changes were evaluated by qPCR. These data confirmed the peptides' ability to penetrate and dismantle biofilm structures.

2.4 Skin Model Evaluation

Ex vivo human skin models were used to assess AMP-mediated effects on inflammation and skin barrier integrity. Cytokines (IL-6, IL-8, TNF-α) were measured via multiplex ELISA. Nitric oxide (NO) production and surface pH were monitored as indicators of microbial metabolic activity and barrier normalization. TEWL and tight junction proteins (claudin-1, occludin) were quantified to evaluate epidermal recovery.

2.5 Microbiome Recolonization and Personalization

Following AMP-driven pathogen clearance, recolonization was performed using *Nitrosomonas eutropha* (AOB), alone or in combination with commensal strains (e.g., *S. hominis*, *L. plantarum*, *L. reuteri*). Colonization dynamics were tracked via 16S rRNA sequencing and qPCR. AI models trained on individual microbiome profiles were used to guide strain selection and dosing. In a double-blind pilot study (n = 25), personalized regimens led to improved symptom resolution and longer-lasting microbial integration compared to fixed-strain controls..

3. Results

3.1 Selective Pathogen Clearance with Hydrolytic Stability

Using our AI-guided peptide discovery platform, we successfully identified lead antimicrobial peptides (AMPs) exhibiting potent activity against key skin pathogens. The selected candidates achieved minimal inhibitory concentrations (MICs) of 1–4 μg/mL against *Staphylococcus aureus* and *Cutibacterium acnes*, while demonstrating excellent selectivity by sparing the commensal *Staphylococcus epidermidis* even at concentrations up to 100 μg/mL.

We further confirmed the functional efficacy of these AMPs through confocal laser scanning microscopy and scanning electron microscopy (SEM), which revealed marked disruption of pathogen biofilms and degradation of the extracellular matrix. To evaluate formulation feasibility and shelf-life potential, we subjected the peptides to simulated topical stress conditions, including sweat, sebum, and proteolytic enzymes (trypsin and chymotrypsin). The AMPs retained structural integrity and antimicrobial function, with stability maintained for up to 24 months under accelerated stability protocols.

These results validate the strength of our discovery platform and highlight the therapeutic potential of our AMP candidates as highly active, selective, safe, and stable components of next-generation dermatological interventions.

3.2 Inflammation Mitigation and Barrier Restoration

We applied our lead AMPs to ex vivo human skin models and observed pronounced anti-inflammatory effects. The treatment significantly reduced pro-inflammatory cytokine levels, including a 65% decrease in interleukin-6 (IL-6), along with notable reductions in IL-8 and TNF-α. At the same time, AMP application elevated nitric oxide production and normalized skin surface pH, both of which are key indicators of a rebalanced skin microenvironment.

We also demonstrated that AMP treatment substantially improved skin barrier function. Transepidermal water loss (TEWL) decreased by 20–35%, indicating enhanced epidermal integrity, while the expression of tight junction proteins—claudin-1 and occludin—was

markedly upregulated at both the mRNA and protein levels. Importantly, we detected no cytotoxicity toward human keratinocytes throughout the 72-hour treatment period, confirming the peptides' safety for topical application

3.3 Rapid and Sustained Recolonization

We initiated recolonization using *Nitrosomonas eutropha* immediately following AMP-mediated pathogen clearance and observed successful colonization on ex vivo skin surfaces within 48 hours. The colonized AOB actively oxidized ammonia into nitrite and nitric oxide, leading to measurable increases in local NO levels and mild acidification of the skin surface (average pH shift from 6.5 to 5.6), both of which contributed to improved skin tone, reduced erythema, and suppression of opportunistic pathogen regrowth.

To further support microbial diversity and ecological resilience, we co-administered additional beneficial strains—including *Staphylococcus hominis*, *Lactobacillus plantarum*, and *L. reuteri*—in specific test arms. These auxiliary microbes enhanced recolonization efficiency and niche occupation, as confirmed by 16S rRNA sequencing and qPCR quantification, which showed increased alpha diversity and restoration of commensal-dominated community structures.

We then evaluated the clinical impact of recolonization in a double-blind pilot study involving 25 participants with mild to moderate skin dysbiosis. Subjects were randomly assigned to receive either AI-personalized formulations—comprising AOB and tailored co-strains—or fixed microbial regimens. Compared to the control group, individuals in the personalized group experienced faster reduction in dryness (mean TEWL decreased by 38% vs. 22%), greater resolution of erythema (72-hour redness index reduction of 41% vs. 19%), and improved subjective comfort scores. Additionally, longitudinal tracking showed that beneficial strains persisted longer on the skin in the personalized group, indicating more stable microbial integration. These findings highlight the unique role of AOB as both ecological stabilizers and functional effectors, and they demonstrate how AI-driven personalization can optimize recolonization strategies to enhance skin recovery and long-term microbiome balance.

4. Discussion

We have developed a two-phase, AI-driven regimen that offers a rational and effective alternative to conventional skin microbiome interventions by integrating selective antimicrobial clearance with functionally guided microbial restoration. This strategy directly addresses the limitations of non-specific antimicrobials and poorly targeted probiotics by combining molecular precision with ecological insight.

In the first phase, we employed our AI-powered peptide discovery platform to identify antimicrobial peptides (AMPs) that exhibit strong activity against common skin pathogens, including *Staphylococcus aureus* and *Cutibacterium acnes.* These peptides demonstrated high specificity, sparing commensal species such as *S. epidermidis*, and effectively disrupted biofilm structures. Their resistance to enzymatic degradation and stability under simulated topical conditions—such as sweat, sebum, and protease exposure—support their potential for real-world dermatological applications. Notably, no cytotoxic effects were observed in keratinocyte models, confirming their safety profile.

In the second phase, we focused on restoring microbial balance through recolonization with beneficial strains. *Nitrosomonas eutropha*, an ammonia-oxidizing bacterium (AOB), served as the primary recolonization agent due to its capacity to modulate skin microenvironment via nitric oxide generation and pH regulation. These functional effects translated into reduced inflammation and improved skin tone. In select conditions, we also applied co-strains such as *S. hominis* and *Lactobacillus spp.*, which enhanced microbial diversity and stability.

To personalize microbial interventions, we incorporated AI algorithms trained on individual 16S rRNA microbiome profiles to design subject-specific formulations. In a controlled pilot study, participants receiving personalized treatments exhibited faster recovery of skin barrier function and greater symptom relief than those receiving fixed-strain regimens. These results highlight the value of integrating diagnostics and personalization into microbiome-based therapies.

Taken together, these two phases form the foundation of a closed-loop microbiome modulation platform that connects high-resolution profiling, targeted antimicrobial treatment, and ecological restoration in a coherent and adaptable framework. The platform is underpinned by three core capabilities: microbial diagnostics with species-level resolution; AI-guided AMP discovery for selective and stable pathogen control; and topical reintroduction of functional microbes for sustained ecological balance.

This integrated approach has broad translational potential. In addition to skincare, we are extending the platform into feminine care through AMP-AOB combination products for mucosal microbiome modulation, and into companion animal health through AOB-based treatments for pet skin dysbiosis. Modular kits integrating diagnostics, AMPs, and personalized probiotics also offer a new path for precision dermatology.

Overall, this work demonstrates the feasibility of a data-driven, ecology-informed approach to microbiome modulation. By unifying detection, treatment, and recovery within a single system, our platform provides a robust and scalable solution for sustainable, individualized skin and mucosal health management.

5. Conclusion

In this study, we have established a robust, AI-driven platform for reconstructing the skin microbiome through a two-step regimen that integrates targeted pathogen clearance with personalized microbial recolonization. By leveraging AI-guided antimicrobial peptide discovery and functional reintroduction of ammonia-oxidizing bacteria, this approach effectively addresses colonization resistance and enables sustainable ecological recovery. The platform's closed-loop design—linking microbial diagnostics, selective AMP intervention, and guided microbial restoration—supports stable, individualized outcomes and demonstrates strong translational potential across multiple domains, including dermatology, mucosal health, and companion animal care.

Future efforts will focus on expanding clinical validation across diverse populations, refining algorithmic personalization, and advancing next-generation formulations that further enhance efficacy, usability, and long-term microbiome resilience.