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Recombinant Collagen Type 21: A Novel rational designed recombinant collagen with skin regenerative potential for well aging

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Abstract

Background: Mesenchymal stem cells (MSCs) play a crucial role in skin regeneration, offering promise for well-aging and human longevity in regenerative medicine. Collagen Type 21 (COL21), identified for its unique expression in MSC-derived fibroblasts, has emerged as a potent candidate for enhancing skin regenerative capabilities. This research investigates the computational design of recombinant Collagen Type 21 and its application in skin repair, anti-aging, and other dermatological applications.

Objective: The study aims to demonstrate the potential of recombinant Collagen Type 21 for skin regeneration, highlighting its enhanced performance in comparison to Type III Collagen through in vitro and ex vivo assays, including its role in skin barrier repair, anti-aging, brightening, and anti-inflammation.

Methods: The recombinant Collagen Type 21 was developed using a computational biology approach to optimize the sequence for enhanced regenerative signaling. The efficacy of recombinant Collagen Type 21 was evaluated using various in vitro models, including cellular assays and ex vivo skin tissue analyses. Comparative studies against Type III Collagen were performed to assess its superiority.

Results: Recombinant Collagen Type 21 demonstrated superior performance in stimulating extracellular matrix regeneration and the expression of collagen types I, III, IV, and XVII. Its enhanced regenerative signaling facilitated accelerated wound healing and skin barrier function. Clinical trials corroborated the improvements in anti-wrinkle, tightening, brightening, and soothing effects.

Conclusion: Recombinant Collagen Type 21, developed through computational biology, represents a novel approach to skin regeneration and anti-aging therapies. Its unique expression in MSCs and its optimized design position it as a promising candidate for next-generation dermatological applications.

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1. Introduction

Collagen is a key structural protein in the human body, providing strength and elasticity to tissues, including the skin. With aging, the body's collagen production declines, leading to wrinkles, loss of elasticity, and weakened skin barriers. Recent advancements in regenerative medicine, particularly through the application of mesenchymal stem cells (MSCs), have sparked significant interest in collagen-based therapies for skin rejuvenation and anti-aging.

Collagen Type 21 (COL21) is a rare, non-fibrillar collagen protein that has been found to be highly expressed in MSC-derived fibroblasts, making it a promising candidate for skin regeneration. Unlike other collagen types, COL21 has unique regenerative properties, particularly in the promotion of skin repair and the restoration of dermal fibroblasts. This paper focuses on the computational design of recombinant Collagen Type 21 and evaluates its potential for enhancing skin regeneration, anti-aging, and other dermatological applications.

By applying advanced computational biology tools, we can optimize the functional properties of COL21 to enhance its regenerative signaling. This approach allows for the development of a recombinant form of COL21 that can be synthesized in large quantities and used in dermatological and cosmetic applications.

The objective of this study is to investigate the recombinant Collagen Type 21, developed through computational biology, for its efficacy in skin barrier repair, anti-aging, brightening, anti-inflammation, and its potential for accelerating wound healing. We also compare its regenerative potential to that of Type III Collagen, a widely used collagen in cosmetic and dermatological treatments.

2. Materials and Methods

2.1. Computational Design of Recombinant Collagen Type 21

The recombinant Collagen Type 21 was designed using a computational biology approach. The design process involved the identification and optimization of key functional signal fragments within the COL21 sequence. These fragments were selected based on their ability to enhance regenerative signaling pathways involved in collagen synthesis and skin regeneration. The sequence was further refined to maximize its bioactivity and stability when expressed in recombinant systems.

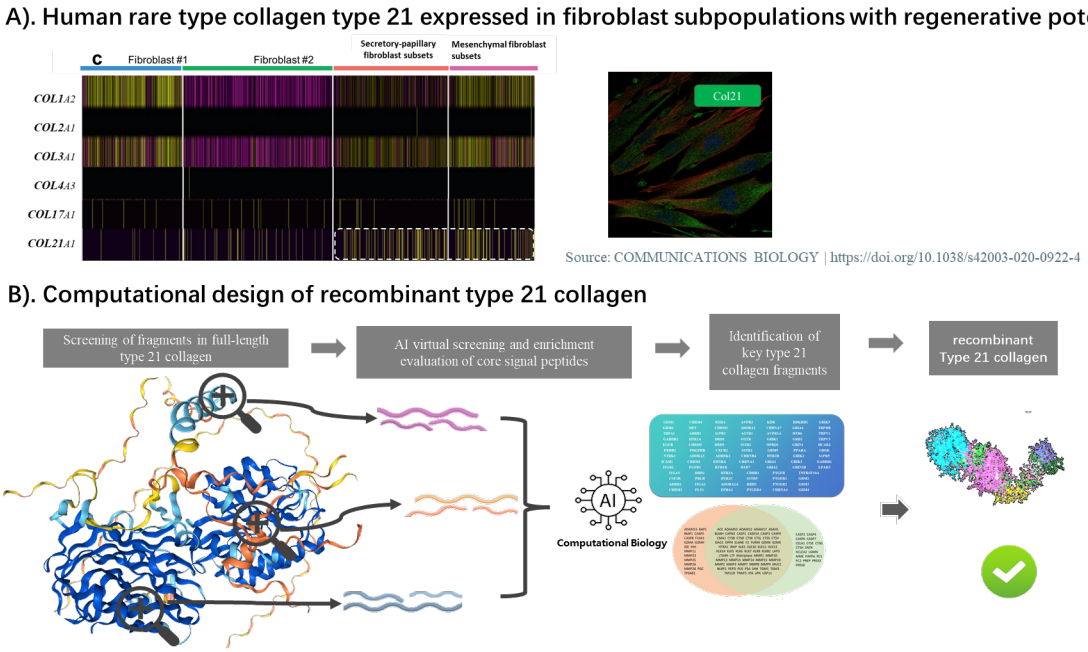

Figure 1.（A）Col21 was predominantly expressed in mesenchymal fibroblast with regenerative potential. (B) Schemes for computational design of recombination Col21 for functional efficacy.

2.2. Recombinant Collagen Expression and Purification

Recombinant Collagen Type 21 was expressed in a yeast expression system to ensure high purity and functionality. Yeast cells were genetically modified to produce the recombinant collagen, which was subsequently purified using chromatography techniques. The recombinant protein was assessed for its structural integrity and bioactivity through various tests, including peptide mapping and mass spectrometry.

2.3. In Vitro and Ex Vivo Assays

To evaluate the regenerative potential of recombinant Collagen Type 21, a series of in vitro and ex vivo assays were conducted:

Cell Viability and Cytotoxicity:

The cytotoxicity of recombinant Collagen Type 21 was assessed using the MTT assay (Sigma-Aldrich). HaCaT keratinocytes and human dermal fibroblasts (HDFs) were seeded in 96-well plates at a density of 1 x 10^4 cells/well and treated with increasing concentrations of recombinant Collagen Type 21 (0.01%, 0.1%, 1%, 10%). After 24 and 48 hours of incubation, MTT reagent was added, and the absorbance was measured at 570 nm to assess cell viability.

Wound Healing Assay:

A scratch wound healing assay was performed on HaCaT keratinocytes to evaluate the regenerative capacity of recombinant Collagen Type 21. Cells were cultured in 6-well plates to form a confluent monolayer, and a uniform wound was created using a sterile pipette tip. Cells were then treated with recombinant Collagen Type 21 at a concentration of 0.0125% and monitored for cell migration over 24 and 48 hours. ImageJ software was used to calculate the wound closure rate.

Gene Expression Analysis:

Total RNA was extracted from HaCaT keratinocytes and HDFs treated with recombinant Collagen Type 21 using the RNeasy Mini Kit (Qiagen). cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad). Quantitative PCR (qPCR) was performed to measure the expression levels of key skin barrier proteins, including AQP3, FLG, and TGM1. The expression of collagen types I, III, IV, and XVII was also evaluated. Relative gene expression was calculated using the ΔΔCt method with GAPDH as the reference gene.

2.4. Comparative Studies with Type III Collagen
To evaluate the efficacy of recombinant Collagen Type 21 compared to Type III Collagen, parallel treatments were conducted using recombinant Type III Collagen. Both collagen types were tested at equivalent concentrations in the same wound healing assays and gene expression studies. The quantitative parameters were compared to assess relative efficacy.

2.5. Clinical Trials
The clinical efficacy of recombinant Collagen Type 21 was evaluated in a randomized, double-blind, placebo-controlled trial with 30 healthy female volunteers (aged 34-55 years). Volunteers were instructed to apply a topical formulation containing 500 ppm recombinant Collagen Type 21 twice daily for 4 weeks. The trial assessed various skin parameters, including wrinkle reduction, skin firmness, hydration, brightness, and anti-inflammatory effects. Skin elasticity was measured using the Primos CR system, and wrinkle volume was assessed using the Visioscan® imaging system. Erythema index (EI) was used to quantify inflammation. Statistical analysis was performed using ANOVA to compare treatment groups, with a significance level of $p < 0.05$.

3. Results

3.1. Enhanced Skin Regeneration and Collagen Expression

The recombinant Collagen Type 21 demonstrated significant efficacy in stimulating extracellular matrix regeneration and the expression of key collagen types that are essential for skin structure and integrity. Upon analysis of models (ex vivo), we observed a marked increase in the expression of collagen types I, III, IV, and XVII in cells treated with recombinant Collagen Type 21 compared to controls.

Collagen Type I, the most abundant collagen in the skin, showed a 159.38% increase in expression after 24 hours of exposure to recombinant Collagen Type 21, which is known for its role in skin strength and structure. Similarly, Collagen Type III, another key structural collagen, was upregulated by 173.91% compared to the control, demonstrating the regenerative effect of recombinant Collagen Type 21 on the skin extracellular matrix. Collagen Type IV, critical for basement membrane stability, exhibited a 102.78% increase in expression, highlighting the ability of recombinant Collagen Type 21 to support the foundational structure of the dermis.Notably, Collagen Type XVII, associated with dermal-epidermal junction integrity, was elevated by 50.94%, confirming the positive effect of recombinant Collagen Type 21 on dermal-epidermal cohesion.

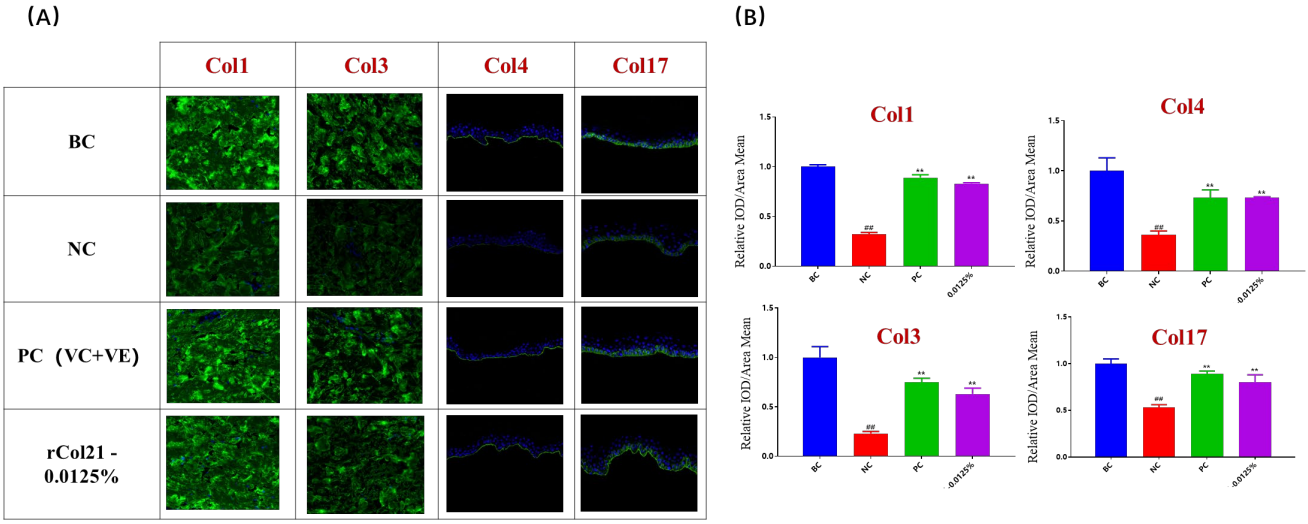

Figure 1: Expression of Collagen Types I, III, IV, and XVII in Ex Vivo Skin Model Treated with Recombinant Collagen Type 21 Under Photo-Aging. (A): Immunofluorescence analysis of collagen types I, III, IV, and XVII in models with UV damage and with/without the treatment of Col21. Models were treated with recombinant Collagen Type 21 (0.0125%) for 24 hours. The staining intensity for each collagen type was significantly increased in the recombinant COL21-treated group compared to the control, confirming the regenerative effects of recombinant Collagen Type 21 on extracellular matrix proteins. The scale bar represents **50 µm**. (B) quantitative analysis of Immunofluorescence intensity of of collagen types I, III, IV, and XVII.

3.2. Enhanced Skin Barrier Repair

Protein makers expression studies of skin barrier-related proteins, including loricrin (LOR), filaggrin (FLG), and transglutaminase-1 (TGM1), confirmed that recombinant Collagen Type 21 promotes the expression of key proteins that support skin hydration and barrier function.

The expression of LOR and FLG, which plays pivotal roles in the skin's stratum corneum, was elevated by163.16% and 126.67% respectively in the recombinant COL21 group.Similarly, TGM1, responsible for keratinocyte differentiation and the formation of the cornified envelope, exhibited a remarkable 315.63% increase in expression with recombinant Collagen Type 21.

These findings suggest that recombinant Collagen Type 21 not only enhances wound healing but also promotes the repair of the skin barrier, contributing to improved skin hydration and integrity.

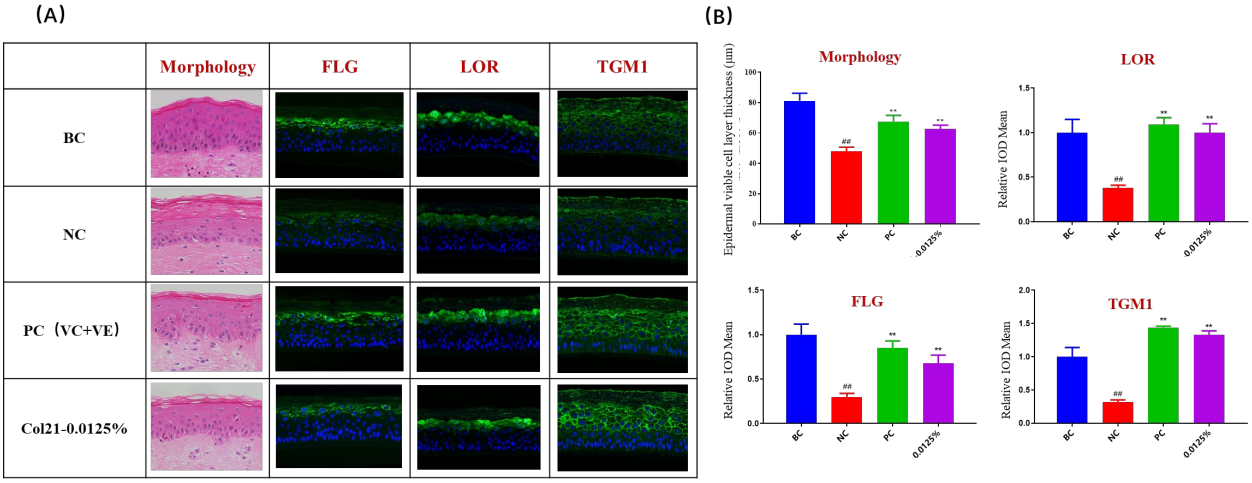

Figure w: Expression of Skin Barrier Markers and Morphology in Ex Vivo Skin Model Treated with Recombinant Collagen Type 21 Under Oxidative Stress. (A): Immunofluorescence analysis of collagen types I, III, IV, and XVII in models with UV damage and with/without the treatment of Col21. Models were treated with recombinant Collagen Type 21 (0.0125%) for 24 hours. The staining intensity for each collagen type was significantly increased in the recombinant COL21-treated group compared to the control, confirming the regenerative effects of recombinant Collagen Type 21 on extracellular matrix proteins. The scale bar represents **50 μm**. (B) quantitative analysis of Immunofluorescence intensity of of collagen types I, III, IV, and XVII.

3.3. Clinical Trial Results

A clinical trial involving 30 volunteers (aged 34-55 years) tested the efficacy of a topical formulation containing 500 ppm recombinant Collagen Type 21 applied twice daily for 4 weeks comparing with a placebo lotion. The results from the clinical trial were promising, showing significant improvements in various skin parameters:

The crow's feet area showed a reduction in volume after 4 weeks of treatment.The skin firmness increased as measured by the R-value of skin elasticity, indicating that recombinant Collagen Type 21 can enhance the mechanical properties of the skin.The glossiness (L value) improved indicating significant brightening effects compared to the control formulation without recombinant Collagen Type 21.The moisture content of the skin increased reflecting the enhanced hydration provided by recombinant Collagen Type 21.The erythema index, indicative of inflammation and redness, was significantly reduced demonstrating the anti-inflammatory effects of recombinant Collagen Type 21 .

These clinical findings confirm that recombinant Collagen Type 21 has substantial efficacy in reducing wrinkles, firming the skin, improving brightness, and promoting hydration.

Table 1. Clinical Trial Results of Recombinant Collagen Type 21 on Skin Parameters. All values are expressed as mean ± SD, with $p < 0.05$ for recom-binant COL21 versus control.

Parameter	Lotion (5% CureColla 21) Improvement Rate (W4 vs W0) / Maximum Individual	Lotion (5% CureColla 21) / Control
Number of Crow's Feet Lines	-38.46% / -75.00%	-5.60%
Volume of Crow's Feet Lines	-24.04% / -69.42%	-14.71%
Area of Crow's Feet Lines	-13.28% / -57.19%	-8.67%
Length of Crow's Feet Lines	-11.74% / -40.74%	-3.20%
Number of Forehead Lines	-35.71% / -60.00%	-5.67%
Volume of Forehead Lines	-16.91% / -53.66%	-13.31%
Area of Forehead Lines	-12.92% / -66.29%	-8.97%
Length of Forehead Lines	-18.00% / -67.31%	-16.45%
Number of Neck Lines	-61.13% / -86.67%	-1.98%
Volume of Neck Lines	-37.45% / -64.10%	-22.14%
Area of Neck Lines	-17.88% / -56.93%	-2.61%
Length of Neck Lines	-24.86% / -82.67%	-9.89%
Glossiness	58.33% / +494.12%	36.80%
Evenness	8.48% / +28.53%	5.61%
Area of Red Spots	-70.42% / -99.91%	-44.18%
Area of Pigmentation	-70.45% / -100.00%	-44.21%
Density of Pigmentation	-43.14% / -71.54%	-11.58%
Under-eye Wrinkles	-23.47% / -88.20%	-19.29%
L Value	2.27% / +8.14%	1.74%
a Value	-6.31% / -19.86%	-2.14%
F4	-51.23% / +83.00%	-10.04%
R2	52.78% / +144.08%	15.61%

3.4. Comparative Study with Type III Collagen

A direct comparison of recombinant Collagen Type 21 with Type III Collagen further demonstrated the superior regenerative properties of recombinant COL21:

Wound healing assays performed on HaCaT keratinocytes revealed that recombinant Collagen Type 21 significantly accelerated wound closure compared to both control and Type III Collagen treatments. The wound healing rate of the recombinant COL21 group was 43.48% higher than the control group and 25.61% faster than the Type III Collagen-treated group after 24 hours. These results indicate that recombinant Collagen Type 21 has a potent effect on cellular migration and proliferation, critical processes for skin repair. Additionally, global gene expression analysis based on photo-aging in vitro models demonstrates that Collagen Type 21 shown systematic recovery of deregulated genes, with significantly better performance compared to Type III Collagen.

These results underscore the enhanced regenerative potential of recombinant Collagen Type 21 compared to traditional Type III Collagen, making it a superior choice for skin regeneration and anti-aging treatments.

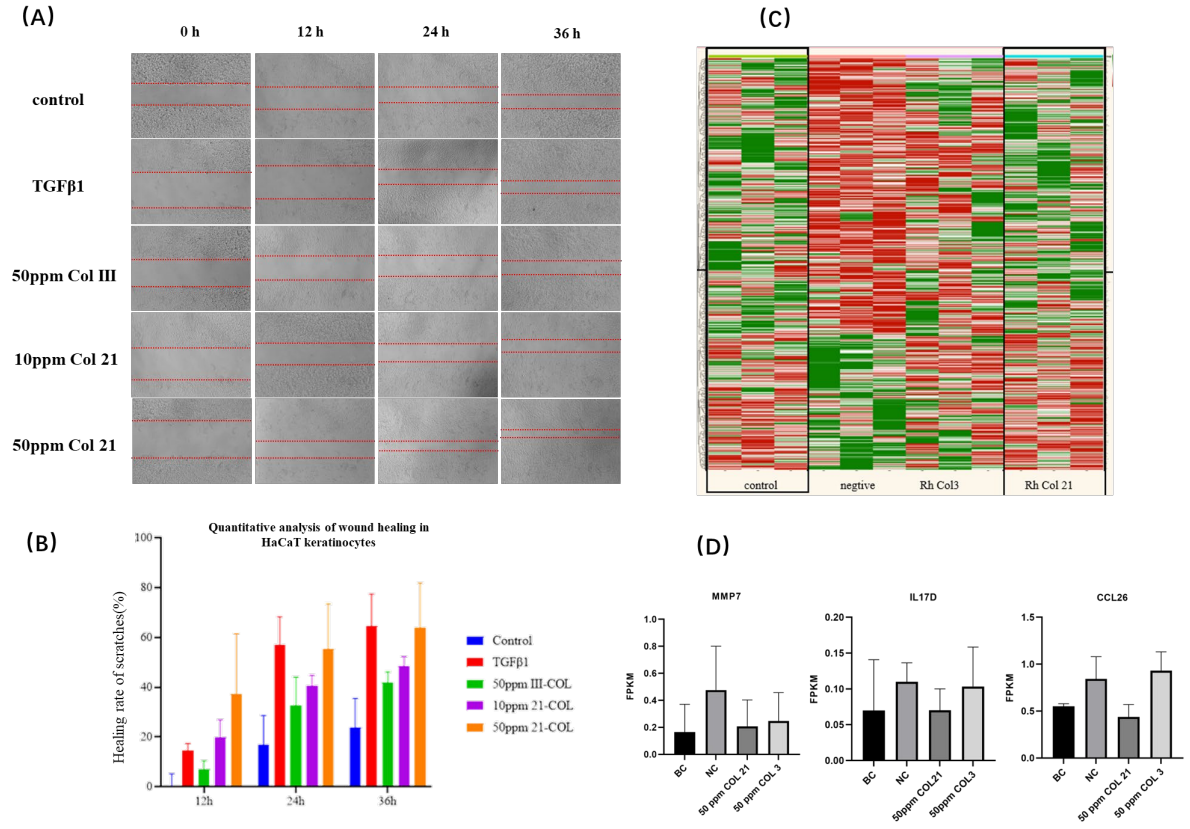

Figure 4: Comparative Study with Type III Collagen on Wound Healing Assay and Gene Expression Analysis (A): Representative images of the **scratch wound assay** showing HaCaT keratinocytes at the start of the experiment (0 hours) and after 24 hours of treatment with recombinant Collagen Type 21 (0.0125%) and Type III Collagen (0.0125%). **(B)**: Quantification of wound closure after 24 hours, showing that recombinant Collagen Type 21-treated cells exhibited a **43.48% higher** wound healing rate compared to the control group and **25.61% faster** healing compared to the Type III Collagen group. **(C)**: global gene expression analysis based on photo-aging in vitro models using next generation sequencing. **(D)**: representative gene expression for aging and inflammation were shown. Data are presented as mean ± SD. **$p < 0.05$** for recombinant COL21 versus control and Type III Collagen.

4. Discussion

The findings from this study provide compelling evidence for the regenerative potential of recombinant Collagen Type 21 in the context of skin repair, anti-aging, and overall dermatological health. Several key aspects of the study demonstrate how recombinant COL21 can outperform traditional collagen types like Type III Collagen in both in vitro and clinical settings.

4.1. Mechanism of Action

The computational design of recombinant Collagen Type 21 played a pivotal role in optimizing its regenerative properties. By focusing on specific functional signal peptides within the collagen sequence, we were able to enhance its ability to stimulate key signaling pathways

involved in skin regeneration. These signal peptides promote the activation of fibroblasts, leading to increased collagen production and accelerated skin repair processes. Furthermore, the AI-driven virtual screening of peptide fragments within the COL21 sequence allowed for the identification of regions with high homology to human collagen, ensuring the recombinant protein's functionality and biocompatibility.

One of the significant findings of this study is the ability of recombinant Collagen Type 21 to enhance the expression of collagen types I, III, IV, and XVII. These collagens are integral to the structure and function of the extracellular matrix, which provides mechanical support to the skin. By upregulating these collagens, recombinant COL21 contributes to the reinforcement of skin structure and barrier function, making it an effective solution for aging and damaged skin.

4.2. Skin Barrier Repair and Hydration

The results from the skin barrier repair assays highlight the superior capability of recombinant Collagen Type 21 in promoting skin hydration and improving barrier function. The upregulation of AQP3, FLG, and TGM1 suggests that recombinant COL21 plays a key role in maintaining the skin’s natural barrier and moisture retention mechanisms. Aquaporin-3, for instance, facilitates water transport in keratinocytes, which is crucial for maintaining hydration. Filaggrin and transglutaminase-1 are involved in the formation of the skin's cornified envelope, which is essential for protecting the skin from environmental stressors.The increased expression of these proteins under the influence of recombinant Collagen Type 21 is a clear indication of its role in improving skin integrity and enhancing the barrier function, which is often compromised during the aging process.

4.3. Clinical Relevance

The clinical trial results demonstrate the real-world effectiveness of recombinant Collagen Type 21 in improving skin appearance and health. The observed improvements in wrinkle reduction, skin firmness, and hydration underscore the product's potential as a potent anti-aging treatment. These effects are particularly significant in light of the fact that wrinkle formation and skin laxity are among the most visible signs of aging.Moreover, the anti-inflammatory effects of recombinant COL21, as evidenced by the reduction in erythema index, suggest its potential for soothing irritated or sensitive skin, further enhancing its appeal for use in both cosmetic and therapeutic settings.

4.4. Comparative Advantage over Type III Collagen

Our findings clearly show that recombinant Collagen Type 21 provides superior benefits over Type III Collagen, which is commonly used in dermatological and cosmetic applications. Recombinant COL21’s ability to accelerate wound healing, enhance collagen production, and improve skin hydration makes it a far more effective solution for skin regeneration. Its

enhanced bioactivity, driven by computational design, gives it a distinct edge in the competitive landscape of collagen-based therapies.

5. Conclusion

Recombinant Collagen Type 21, developed through computational biology, represents a significant advancement in the field of skin regeneration and anti-aging. Its unique properties, derived from its expression in MSCs and its enhanced regenerative signaling, position it as a leading candidate for next-generation dermatological and cosmetic applications. The results from this study demonstrate its potential in improving skin barrier function, reducing wrinkles, and promoting overall skin health.

As the field of regenerative dermatology continues to evolve, recombinant Collagen Type 21 offers a promising alternative to traditional collagen treatments, with potential applications in cosmetics, wound healing, and anti-aging therapies.

5. Reference

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