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"RNA Interference in Cosmetic Innovation: Advancing Skin Whitening, Anti-Aging, Hypoallergenic Properties, and Beauty Enhancement"

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1. Introduction

For centuries, cosmetic formulations have relied on traditional active ingredients such as vitamins (C and E), peptides, hyaluronic acid, and retinoids to improve skin appearance and address dermatological concerns.[1, 2] While these compounds offer some benefits, they often lack sufficient efficacy, specificity, or safety—particularly for individuals with sensitive skin or complex skin conditions.[3, 4] As consumer expectations rise and scientific advancements accelerate, there is a growing demand for next-generation actives that are both pecise and biocompatible.[5]

RNA interference (RNAi) has emerged as a transformative molecular biology tool that enables highly specific gene silencing.[6] By employing short RNA molecules to target and degrade messenger RNA (mRNA), RNAi effectively blocks the production of undesired proteins at the post-transcriptional level.[7] This paradigm-shifting approach, recognized by Nobel Prizes in 2006 and 2024, holds immense therapeutic potential and is now gaining traction as a novel strategy in skincare.[8] In cosmetic applications, RNAi offers precise modulation of gene expression in skin-related pathways, enabling targeted treatment of aging, pigmentation, inflammation, and other complex skin concerns.[9]

However, siRNA faces significant challenages as a consmetic ingredient due to its poor stability and limited ability to penetrate the skin barrier without effective delivery systems.[10] To address these limitations, we have developed an innovative skin delivery and RNA modification platform specifically designed to enhance the transdermal transport of RNA molecules. This platform has been used to developed RNAi-based ingredients targeting key

concerns such as skin brightening, anti-aging, and hypoallergenicity, offering more personalized and effective skincare solutions.

For our skin brightening ingredient, tyrosinase (TYR) mRNA was selected as the target due to its pivotal role as the enzyme that governs melanin biosynthesis in melanocytes (Figure 1a). As the rate-limiting catalyst, TYR facilitates two critical oxidative reactions: the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), followed by the oxidation of L-DOPA to dopaquinone.[11] These initial steps are essential in the synthesis of eumelanin and pheomelanin, the primary pigments responsible for skin, hair, and eye color.[12]

For our anti-aging ingredient, matrix metalloproteinase mRNA was selected as the target due to its central role in extracellular matrix (ECM) degradation, particularly in the breakdown of fibrillar collagens (types I and III), which are critical for maintaining the structural integrity of the skin (Figure 1b). [13]

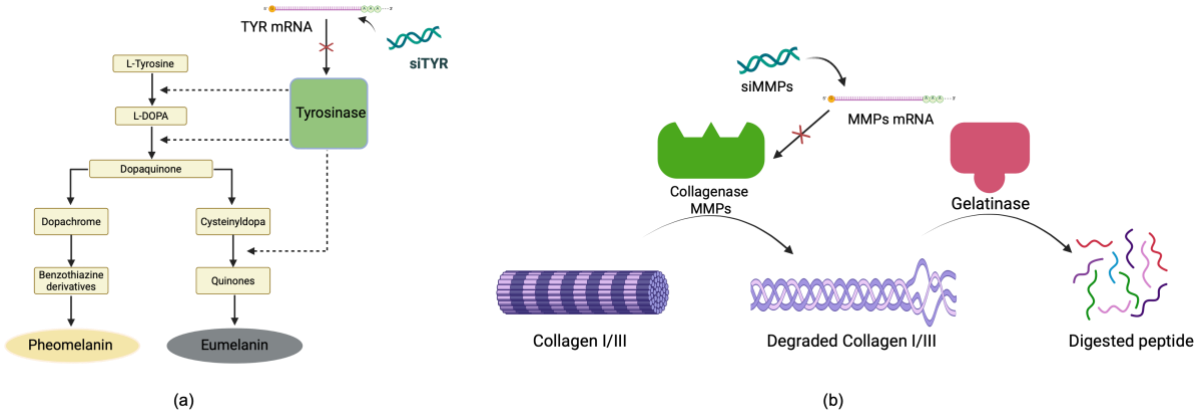

Figure 1. siRNA targets for cosmetic applications in skin brightening and anti-aging. (a) Melanin biosynthesis pathway in melanocytes, where tyrosinase (TYR) is targeted by siTYR to downregulate melanin production, resulting in skin brightening effects. (b) Degradation of fibrillar collagens (types I and III) by matrix metalloproteinases (MMP), siMMPs targets MMPs mRNA to inhibit collagen breakdown, thereby preserving skin structure and reducing visible signs of aging.

2. Materials and Methods

RNAi Compouds Synthesis

All chemicals were purchased from commercial supplier and used with no further purification. Sequences targeting human tyrosinase (hTYR, NM_000372.5) and human matrix metalloproteinase (hMMPs) were identified using our AI platform. RNA oligonucleotides were synthesized via standard phosphoramidite chemistry on a LK-48M synthesizer (Jiangsu Lingkun Biotechnology Co., Ltd.). After synthesis, oligonucleotides were cleaved from the support and deprotected using ammonium hydroxide. The crude products were purified by reverse-phase HPLC (Thermo Fisher) and then analyzed for purity and molecular weight using HPLC and ESI-MS (Thermo Fisher).The purified sense and antisense strands were diluted in RNase-free water. Equimolar amounts of each strand were mixed and heated 90 °C for 15 min, then allowed to cool slowlly to room temperature to facilitate annealing. The integrity of the resulting siRNA duplexes was confirmed by MS. All annealed siRNAs were lyophilized and stored at -20 °C.

Cell Culture and siRNA Treatment

Human A375 melanoma cell and human HT1080 fibrosarcoma cells were purchased from Cell bank/Stem Cell Bank, Chinese Academy of Sciences. Both cell lines were cultured in

Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS, Gibco) and maintained at 37 °C with 5% CO_2 (Thermo Fisher Scientific).
For siRNA treatment of cells, cells were seeded into 12-well plate at a density of 2×10^5 cells per well. siRNA compounds were added at final concentrations of 10 μM. Cells were incubated for 48 hours before to collection for downstream analyses.
For the human HT1080 fibrosarcoma cells, the model group and treatment group were washed gently 1–2 times with D-Hanks solution after removing the culture medium. The cells were then subjected to UVA irradiation (9 J/cm²) to establish a photoaging model.

Determinnation of siRNA Gene silencing by RT-qPCR

Total RNA was extracted from treated and control cells using TRIzol™ Reagent (Invitrogen) according to the manufacturer's protocol. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific), and 1 μg of total RNA was reverse transcribed into complementary DNA (cDNA) using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara Bio, Japan).
Quantitative real-time PCR (RT-qPCR) was performed using TB Green® Premix Ex Taq™ II (Takara Bio, Japan) on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, USA). Specific primers were designed for TYR, MMPs, and the housekeeping gene GAPDH (used as an internal control). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Each reaction was run in triplicate, and all experiments were repeated at least three times independently.

Reduction of Pigmentation and Prevention of Collagen I/III Degradation by siRNA

Melanin Content Assay

Following siTYR treatment, A375 cells were washed with PBS and lysed in 1 N NaOH containing 10% DMSO at 80 °C for 1 hour to solubilize melanin. The melanin content was quantified by measuring absorbance at 400 nm using a microplate reader. Melanin levels were normalized to total protein content, determined by BCA assay.

Collagen I/III Quantification by Western Blotting

To assess the effect of siMMPs on collagen preservation, HT1080 cells were lysed using RIPA buffer containing protease and phosphatase inhibitors (Beyotime, China). Total protein concentration was measured using a BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of protein (20–30 μg) were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore). Membranes were blocked with 5% non-fat milk in TBST buffer for 1 hour at room temperature, followed by overnight incubation at 4 °C with primary antibodies against collagen I (e.g., Abcam, ab34710), collagen III (e.g., Abcam, ab7778), MMPs (e.g., CST, #38929), and β-actin (loading control; e.g., Proteintech, 66009-1-Ig).
After washing, membranes were incubated with HRP-conjugated secondary antibodies (1:5,000 dilution) for 1 hour at room temperature. Bands were visualized using enhanced chemiluminescence (ECL) reagents (Bio-Rad) and imaged on a ChemiDoc MP Imaging System (Bio-Rad). Densitometric analysis was performed using ImageJ software, and expression levels of collagen I/III were normalized to β-actin.

3. Results

Evaluation of siTYR in vitro

To assess the activity of siTYR (10 uM) on melanin production, it was tested in the A375 melanoma model to evaluate the knockdown of TYR mRNA levesl and the reduction of melanin content. Treatment of A375 cells with siTYR led to a significant decrease in hTYR mRNA levels, as shown in Figure 2a. Quantitative PCR analysis revealed that siTYR effectively silenced TYR gene expression compared to the control group. In Figure 2b, melanin production was monitored 6 days after siRNA treatment. A notable decrease in melanin content was

observed in siTYR-treated cells, along with visible signs of cell decomposition, indicating reduced pigmentation associated with TYR knockdown.

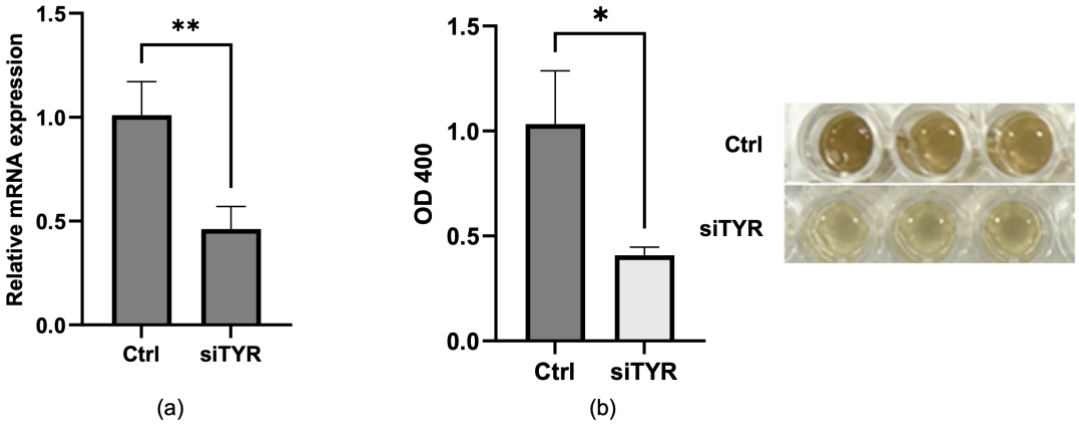

Figure 2. hTYR gene silencing and reduction of melanin content by siTYR in A375 model. (a)% hTYR mRNA expression remaning after treatment of siTYR; (b) Melanin content was monitored after 6 days of siTYR treatment. Representative image showing decomposed cells and reduced pigmentation.

Evaluation of siMMPs in Vitro

To evaluate the activity of siMMPs (10 μM) on MMPs expression and extracellular matrix remodeling, experiments were conducted using the human HT1080 fibrosarcoma cell model. After UVA irradiation, HT1080 cells were treated with siMMPs. Quantitative PCR analysis revealed that a significant reduction in MMPs mRNA levels following siMMPs treatment compared to the UVA model group (Figure 3a), confirming effective gene silencing. Additionally, protein expression of MMPs, collagen I/III were assessed 2 days after siRNA treatment via Western blot (Figure 3b). An increase in collagen deposition was observed in siMMPs-treated cells, suggesting that MMPs knockdown reduced collagen degradation and contributed to the preservation of the extracellular matrix.

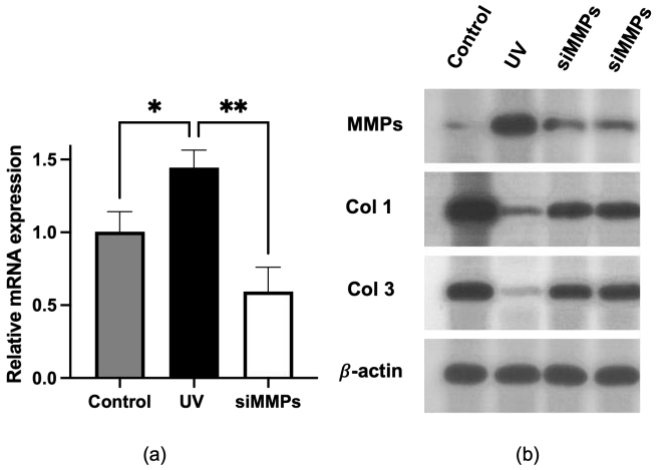

Figure 3. hMMPs gene silencing and collagen preservation by siMMPs in UVA irradiated HT1080 model. (a) % hMMPs mRNA expression remaining after treatment with siMMPs following UVA irradiation; (b Representative Western blot images showing the protein levels of MMPs, collagen I, and collagen III 2 days after siMMPs treatment. Protein expression was normalized to β-actin.

Clinical Evaluation of siTYR for skin brighting

A 28-day clinical study was conducted to assess the skin-whitenning efficacy of a cosmetic formulation containing siTYR. Ten healthy volunteers applied the product topically to the skin on the back that has been darkened by UV exposure daily. Skin assessments were performed at baseline and after 28 days of use, ITA° value, melanin index value, and visual skin color score were quantitatively evaluated (Table 1).

Following 28 days of treatment, siTYR demonstrated marked improvement rates (IR) of 10.81% for ITA°, 9.77% for MI, and 30.34% for the VACS compared to baseline (D0), showing significant differences ($P < 0.005$ for all parameters). The results showed that siTYR effectively inhibited the expression of TYR. At the upstream stage, siTYR reduced the generation of eumelanin and pheomelanin by inhibiting the production of dopaquinone, thereby achieving the effect of skin-whitening. In addition, in a human skin patch test, all 30 subjects exhibited negative cutaneous reactions. These results conclusively validate the human safety profile and functional efficacy of the siTYR.

Table 1. Cosmetic Depigmentation Efficacy Assessment Resluts After 28 Days of Product Use

Parameter	Test Time Point	Mean	Standard Deviation	Product Usage Compared to D0	P Value	Significance
ITA°	D0	28.93	2.00	/	/	/
	D28	32.06	2.19	10.81%	< 0.001	**
Melanin Index	D0	205.80	18.08	/	/	/
	D28	185.70	14.87	9.77%	< 0.001	**
Visual Skin Color Score	D0	8.9	0.9	/	/	/
	D28	6.2	0.4	30.34%	= 0.004	**

Data analysis was performed using SPSS 25.0 statistical software. Paired t-tests or Wilcoxon signed-rank tests were used to evaluate differences between baseline (D0) and follow-up (D28) values for each parameter. Statistical significance was denoted as follows: "n.s." for no significant difference, "*" for $p < 0.05$, "**" for $p < 0.01$. A p-value of < 0.05 was considered statistically significant, < 0.01 highly significant.

Clinical Evaluation of siMMPs for Anti-Wrinkle and Firming Efficacy

A 28-day clinical study was conducted to assess the anti-wrinkle and firming efficacy of a cosmetic formulation containing siMMPs. Twelve healthy volunteers applied the product topically to the facial skin twice daily. Skin assessments were performed at baseline and after 28 days of use, utilizing high-resolution imaging and cutometry to quantitatively evaluate wrinkle depth, skin elasticity, and firmness (Table 2).

After 28 days of product use, the R2 value of canthus skin elasticity significantly increased by 16.13% compared to baseline (D0), indicating a notable improvement in skin firmness ($p < 0.01$). In addition, the number of eye wrinkles decreased by 20.10%, a reduction that was statistically significant according to analysis of variance ($p < 0.05$). Furthermore, the wrinkle area in the canthus region decreased by 15.80% at Day 28 compared to baseline, also showing a significant difference ($p < 0.01$). Overall, these findings suggest that siMMPs effectively inhibits MMPs expression, thereby reducing collagen degradation, promoting extracellular matrix preservation, and contributing to visible improvements in skin texture and firmness.

Table 2. Changes in Canthus Skin Elasticity and Wrinkle Parameters After 28 Days of Product Use

Parameter	Test Time Point	Mean	Standard Deviation	Product Usage Compared to D0	P Value	Significance
R2 Value (Skin Elasticity)	D0	0.62	0.09	/	/	/
	D28	0.72	0.08	16.13%	< 0.01	**
Number of Eye Wrinkles	D0	92.83	38.22	/	/	/
	D28	74.17	33.52	-20.10%	< 0.05	*
Canthus Wrinkle Area	D0	67.91	12.18	/	/	/
	D28	57.18	14.33	-15.80%	< 0.01	**

Data analysis was performed using SPSS 25.0 statistical software. Paired t-tests or Wilcoxon signed-rank tests were used to evaluate differences between baseline (D0) and follow-up (D28) values for each parameter. Statistical significance was denoted as follows: "n.s." for no significant difference, "*" for $p < 0.05$, "**" for $p < 0.01$. A p-value of < 0.05 was considered statistically significant, < 0.01 highly significant.

4. Discussion

The present study evaluated the efficacy of siRNA-based cosmetic formulations, specifically focusing on siTYR for skin brightening, and siMMPs for anti-wrinkle and firming effects. These results highlight the potential of RNA interference (RNAi) technology as a novel, targeted approach in addressing common dermatological concerns, such as pigmentation and aging, offering precise and biocompatible solutions.

Targeting tyrosinase (TYR) mRNA with siTYR demonstrates strong potential for regulating melanin biosynthesis, the primary pathway responsible for skin pigmentation. In vitro studies using A375 melanoma cells showed a significant decrease in melanin content, accompanied by downregulated TYR gene expression. These cellular results were further supported by a 28-day clinical study, where topical application of siTYR led to marked improvements in ITA° (+10.81%), melanin index (–9.77%), and visual skin color score (–30.34%), all with statistical significance ($P < 0.005$). Moreover, no adverse skin reactions were observed in a human patch test with 30 subjects. Together, these findings confirm both the efficacy and safety of siTYR as a targeted and well-tolerated skin-brightening agent.

siMMPs, on the other hand, demonstrates significant anti-aging effects by reducing wrinkle depth and improving skin elasticity and firmness. The 28-day clinical study showed a 16.13% improvement in the R2 value of canthus skin elasticity ($p < 0.01$), indicating a marked improvement in skin firmness. Additionally, the reduction in the number of eye wrinkles by 20.10% and the significant decrease in canthus wrinkle area (15.80%) further support the product's anti-aging potential. These results align with previous research on the role of matrix metalloproteinases (MMPs) in collagen degradation, emphasizing the importance of inhibiting MMPs expression to preserve collagen integrity and maintain skin structure.

Beyond these products, additional siRNA-based ingredients are under development to further expand the use of siRNA technology in targeting various aspects of skin health, including oil control, inflammation, and more.

Despite the promising results, challenges remain for RNAi-based cosmetic formulations, primarily regarding the stability and skin penitraition of siRNA molecules.[14, 15] While our

delivery platform in this study improves transdermal transport, further refinement is needed to enhance efficiency and stability. Additionally, long-term safety and efficacy require evaluation in larger, diverse polpulations. Future research could explore combining siMMPs and siTYR in one formulation to address both aging and pigmentation. Investigating siRNA's potential to target other skin-related genes could further expand its cosmetic applications.

5. Conclusion

In conclusion, the application of siRNA-based technologies, specifically targeting TYR and MMPs, shows great promise in advancing the field of cosmetics by providing precise, effective, and safe solutions for skin concerns such as aging and pigmentation. This study highlights the potential of RNA interference as a cutting-edge molecular approach to skincare, supported by an advanced delivery platform. As the beauty industry continues to demand more efficient and personalized treatments, RNAi technology offers a transformative approach to achieving healthier, more youthful skin.

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