American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

The Clinical Efficacy of Recombinant Type III Collagen Combined with Monopolar Radiofrequency Device on Anti-Aging of Facial Skin

Huanmei Dai[1], Shentong Wang[1], Mengyu Yang[1], Jianlan Liu[1], Chengyu Wu[1], Qiongya Li[1], Wensu Fan[1], Jing Zhang[1] and Jie Yu[1,2]*

[1]*R&D centre, Sichuan Liyangongfang Biotechnology Co., Ltd., Meishan 620000, Sichuan, China*

[2]*School of Bioscience and Technology, Chengdu Medical College, Chengdu,610500, Sichuan, China*

***Corresponding author:** Jie Yu, yujierolor@hotmail.com, R&D centre, Sichuan Liyangongfang Biotechnology Co., Ltd., Meishan 620000, School of Bioscience and Technology, Chengdu Medical College, Chengdu,610500, Sichuan, China.

To Cite This Article: *Huanmei Dai, Shentong Wang, Mengyu Yang, Jianlan Liu, Chengyu Wu, et al. The Clinical Efficacy of Recombinant Type III Collagen Combined with Monopolar Radiofrequency Device on Anti-Aging of Facial Skin. Am J Biomed Sci & Res. 2025 26(4) AJBSR.MS.ID.003458,*

DOI: 10.34297/AJBSR.2025.26.003458

Received: March 24, 2025; **Published:** April 04, 2025

Abstract

Background: Skin aging is caused by the combined effects of endogenous and exogenous factors, characterized by reduced collagen synthesis, collagen loss and fragmentation, damage to the collagen fiber network, and resultant decreases in elasticity, sagging, roughness, and wrinkles. Recombinant collagen, prepared via DNA recombination technology, consists of amino acid sequences or functional fragments encoded by human collagen genes. It replenishes lost collagen, repairs damaged collagen fiber networks, and promotes fibroblast migration and repair. Monopolar Radiofrequency (RF) delivers current through electrodes, generates heat via skin impedance, volumetrically heats the dermis, induces reversible thermal damage, activates tissue repair mechanisms, promotes collagen neogenesis, and reshapes fiber structures, thereby improving skin sagging and restoring elasticity.

Methods: In this study, 33 subjects underwent four combined treatment sessions with recombinant type III collagen and monopolar RF, administered once weekly. Skin indicators were evaluated using instruments such as Visia-CR, and subjective assessments were statistically analysed before treatment and on days 7, 14, 21, and 28 post-treatments.

Results: The results showed significant improvements in skin elasticity, firmness, stratum corneum moisture content, skin colour, brightness, gloss, and the area ratios of nasolabial folds and canthal wrinkles after each treatment, with improvement rates increasing with treatment frequency ($p < 0.05$). The area ratio of under-eye wrinkles improved significantly on day 14 ($p < 0.05$).

Conclusions: This study confirmed that the combined treatment of recombinant type III collagen and monopolar RF is effective for facial anti-aging, with no reported adverse reactions and high subject satisfaction. It provides a new feasible method for facial anti-aging.

Keywords: Recombinant type III collagen, Monopolar radiofrequency, Combined therapy, Facial anti-aging

Introduction

Collagen constitutes approximately 70% of the dry weight of human skin and serves as a principal component of the extracellular matrix, playing a pivotal role in maintaining skin structural integrity [1]. Among the various collagen types, Type I and Type III collagen are the predominant fibrous collagens in the skin. Type I collagen, being the most abundant fibrous collagen in the dermis, endows the skin with toughness and provides structural support. In contrast, Type III collagen, while less abundant, supports the skin

and imparts tensile strength, thereby conferring elasticity and a delicate, soft texture upon the skin [2].

Notably, the proportion of Type III collagen in the skin undergoes significant changes with age. In infant skin, Type III collagen accounts for 50% of the total collagen content, whereas in adult skin, its proportion decreases to only 15% - 20% [3]. Adults are incapable of synthesizing Type III collagen through dermal fibroblasts; instead, they rely on fibroblasts originating from the bone marrow for its production. Consequently, the level of Type III collagen in the skin gradually declines with each passing year [4].

The overall collagen content in the skin is also affected by age and external factors. It has been reported that the total collagen content in the skin decreases at an annual rate of approximately 1% [5]. Alterations in the ratio of Type I to Type III collagen in the skin can trigger a range of aging - related issues. These include facial skin collapse, loss of skin elasticity, deepening wrinkles, enlarged pores, uneven skin tone, water - oil imbalance, and dryness and roughness of the skin [6]. Therefore, it is necessary to supplement the skin with exogenous Type III collagen to combat the aging process.

Traditional animal - derived collagen, however, has several drawbacks. These include the potential for immune rejection reactions, weak efficacy, low purity of the collagen mixtures, and the risk of transmitting animal - sourced viruses and infectious diseases [7]. In contrast, recombinant Type III collagen is produced using DNA recombination technology. By optimizing and re expressing the original amino acid sequence of human Type III collagen, recombinant Type III collagen can be manufactured. This type of collagen boasts high biocompatibility, high bioactivity, high safety, and lacks immunogenicity, making it an excellent ingredient for anti - aging skincare products [8].

In addition to daily skincare, medical aesthetic technologies such as Radio Frequency (RF), laser, and ultrasound have become common methods for preventing and treating facial aging [9]. RF technology has gained widespread application due to its non-invasive nature, safety, efficacy, absence of significant adverse reactions, and its melanocyte-sparing mechanism, which reduces the risk of pigmentation [10]. The therapeutic effects of RF are divided into immediate and long-term effects: the immediate effect involves heating the dermis and subcutaneous tissue, leading to instant improvement in skin laxity; the long-term effect is attributed to controlled thermal injury that activates the skin's repair mechanisms, upregulates type I collagen mRNA expression, promotes the synthesis of new collagen fibers, and induces collagen remodelling, a process that occurs 2-6 months after RF treatment and has a prolonged duration [11,12]. Based on the number and distribution of electrodes, RF energy delivery methods are classified into four types: monopolar, bipolar/multipolar, multichannel, and fractional RF, with monopolar RF further categorized into closed-circuit monopolar, open-circuit monopolar, and bipolar-focused monopolar systems [10,12]. Closed-circuit monopolar RF uses a contact electrode and a grounding electrode to transmit current, which generates heat through tissue impedance, achieving volumetric heating of the dermis and improving skin laxity through both immediate and long-term thermal effects [12].

Based on the aforementioned background, this study employed a return-current monopolar Radio Frequency (RF) device in conjunction with recombinant type III collagen for application on the facial skin of participants. The efficacy of this regimen in combating facial skin aging was evaluated through the analysis of various skin parameters post-treatment and the statistical outcomes of participants' subjective assessments. The results demonstrated that the combined use of monopolar RF and recombinant type III collagen significantly improved the anti-wrinkle, moisturizing, firming, and skin-brightening effects on facial skin.

Materials and Methods

Reagents and Instruments

Experimental Samples: The formulations (These contain recombinant type III collagen): essence, lyophilized bioactive powder, sterile reconstitution solution, facial mask, collagen facial cream, Sichuan Liyangongfang Biotechnology Co., Ltd.; Recombinant type III collagen, Shanxi Jinbo Biopharmaceutical Ltd.

Experimental Instruments: E-BOX Monopolar RF Beauty Device, Beijing Guanzhou Technology Co., Ltd; Skin elasticity tester Cutometer dual MPA580, Skin moisture tester Corneometer CM825, Skin colour testing probe Colorimeter CL400, Skin glossiness testing probe Glossimeter GL200, Courage+Khazaka, Germany; Facial image analyzer VISIA-CR, CANFIELD, USA.

Subjects and Inclusion/Exclusion Criteria

A total of 33 healthy Chinese male and female subjects, aged between 25 and 53 years, who met the inclusion and exclusion criteria were enrolled in the study. Among them, 32 were female and 1 was male, with an average age of 42.00 ± 1.53 years.

Inclusion Criteria: Dry facial skin and lack of moisture, with poor barrier function and dull skin;

Self-identified sensitive skin, prone to facial redness;

Laxity and lack of elasticity of facial skin;

The subjects have obvious forehead wrinkles and nasolabial folds, fine lines or wrinkles on the face, and wrinkles at the corners of the eyes that conforms to SKIN AGING ATLAS levels 1 to 5 [13];

The subjects can cooperate well with the experiment and maintain a regular lifestyle during the experiment period;

The subjects are able to read and understand all the contents of the informed consent form, and sign the informed consent form voluntarily;

The subjects agree not to use any cosmetics, drugs, or health products that may affect the results during the experiment period.

Exclusion Criteria: Skin diseases in the experiment sites that may affect the judgment of the experiment results;

Highly allergic individuals;

Females who are pregnant, breastfeeding or planning to become pregnant during the experiment period;

Individuals with severe heart, liver, and kidney dysfunction, as well as severe immune dysfunction;

Individuals with mental illness, severe endocrine disorders, and oral contraceptive pills users;

Participants in drug clinical trials or other trials within 30 days, or those who have systematically used drugs that affect the trial results within the past week;

Individuals who have consumed or applied beauty products orally or topically within 2 weeks that may affect the experiment results;

The researcher deems that it is not suitable to participate in this study;

Individuals with large metal implants, high fever with internal heat, and those with pacemakers are prohibited from undergoing the experiment;

Other corresponding exclusion criteria.

Testing Environment

Environmental temperature 20.5℃ -21.9℃, humidity 48.0% RH-58.0% RH.

Testing Indicators

Subjective Evaluation Questionnaire [14-15]

(Table 1)

Table 1: Subjective Evaluation Questionnaire.

Evaluation indicators	After use, the forehead wrinkles have been improved
	After use, the nasolabial folds have been improved
	After use, the crow's feet have been improved
	After use, it can brighten facial skin tone
	After use, the skin's hydration have been improved
	After use, the elasticity of facial skin has been improved
	After use, the tightness of facial skin has been improved
	After use, the facial skin becomes more radiant
	This treatment is mild and non-irritating

Objective testing indicators [16-17]

(Table 2)

Table 2: Objective testing indicators.

Detection site	Indicator	Performance evaluation indicator	Instrument
Cheek (Left/ Right)	Skin Elasticity R2	firming	Skin elasticity tester Cutometer dual
	Skin Firmness F4		MPA580
	Stratum Corneum Hydration	hydrating	Skin moisture tester Corneometer CM825
	Skin Color ITA° Value Skin Color L*Value	brightening	Skin color testing probe Colorimeter CL400
	Skin Glossiness Parameter	brightening	Skin glossiness testing probe Glossymeter GL200
Full face (Left Center Right)	VISIA-CR Under-Eye Wrinkle Area Percentage	anti-wrinkle	Facial image analyzer VISIA-CR
	VISIA-CR Skin Glossiness Parameter	brightening	
	VISIA-CR Nasolabial Fold Area Percentage	anti-wrinkle	
	VISIA-CR Canthal Wrinkle Area Percentage		

Experimental Methods and Procedures

Instructions for Using Experimental Samples: After cleaning the face with warm water, introduce 3g essence into the face with E-BOX Monopolar RF until it is completely absorbed;

Clean the subject's face with pre-moistened cleansing wipes, add 1 bottle of sterile reconstitution solution to 1 bottle of lyophilized bioactive powder to dissolve completely, and use an E-BOX monopolar RF to introduce the mixture onto the subject's face until it is completely absorbed;

Clean the subject's face with a damp facial cleanser, and apply one side of the mask to the subject's face for 10-15 minutes;

After taking the following film, clean the face of the subject with a wet face cleaning towel, and apply 0.5g collagen face cream on the face of the subject and gently pat it until it is absorbed.

Experimental Process:

A) **Subject's first visit (D0):** The subject uses facial cleansing products to clean their face and dries their skin with a dry tissue. After sitting quietly for 30 minutes in a laboratory with a temperature of 21°C± 1°C and a humidity of 50%RH ± 10%RH, the laboratory technician collects the subject's facial skin baseline data according to Table 1; Subsequently, professional technicians will guide the subjects to complete the experiment according to the steps shown in section 2.5.1.

B) **Follow up of subjects (D7, D14, D21):** Subjects D7, D14, and D21 will be followed up on time, using facial cleansing products to clean their faces and dry their skin with dry wipes. After sitting quietly for 30 minutes in a laboratory with a temperature of 21 °C± 1°C and a humidity of 50%RH ± 10%RH, laboratory technicians will collect data on subjects' facial skin D7, D14, and D21 according to Table 1; Subsequently, professional technicians will guide the subjects to complete the experiment according to the steps shown in section 2.5.1.

C) **Subject Follow up (D28):** Subject D28 will be followed up on time, using facial cleansing products to clean their face and dry their skin with a dry tissue. They will sit quietly for 30 minutes in a laboratory with a temperature of 21°C± 1°C and a humidity of 50%RH ± 10%RH, and complete the self-assessment questionnaire shown in 2.4.1. The laboratory technician will collect data on the subject's facial skin D28 according to (Table 1).

Data Statistics and Analysis Methods

Apply statistical analysis software for data statistical analysis. If the values are normally distributed, the T-test method (paired sample T-test) is used for statistical analysis; If the values are non-normally distributed, the Wilcoxon signed rank test (Wilcoxon signed rank test) is used for statistical analysis; The statistical methods all use a two tailed test, with a test level of α=0.05.

Questionnaire result statistics method: Calculate the proportion of people with a score ≥ 4; Use binomial distribution method for statistical analysis, with an expected value set at 0.5 and a significance level of α=0.05. If the $P < 0.05$, it indicates a significant difference.

Significance annotation method: "n.s" indicates no statistical difference, $P \geq 0.05$; $P < 0.05$ indicates a significant difference ("*" represents $0.01 \leq P<0.05$; "**" represents $0.001 \leq P< 0.01$; "***" represents $P < 0.001$).

The safety evaluation adopts statistical description method to analyze the degree and duration of adverse events case-by-case.

The formula for calculating the rate of change is as follows:

Using the experimental product for 7 days △ (difference)=D7-D0

Using the experimental product for 14 days △ (difference)=D14-D0

Using the experimental product for 21 days △ (difference)=D21-D0

Using the experimental product for 28 days △ (difference)=D28-D0

The Change rate of experimental product for 7 days = $\frac{\text{Mean(D7)-Mean(D0)}}{\text{Mean(D0)}} \times 100\%$

The Change rate of experimental product for 14 days = $\frac{\text{Mean(D14)-Mean(D0)}}{\text{Mean(D0)}} \times 100\%$

The Change rate of experimental product for 21 days = $\frac{\text{Mean(D21)-Mean(D0)}}{\text{Mean(D0)}} \times 100\%$

The Change rate of experimental product for 28 days = $\frac{\text{Mean(D28)-Mean(D0)}}{\text{Mean(D0)}} \times 100\%$

In the formula,

D0-Basal values of skin parameters in the subject area before application of the experimental product.

D7-Values of skin parameters in the subject area using the experimental product for 7 days.

D14-Values of skin parameters in the subject area using the experimental product for 14 days.

D21-Values of skin parameters in the subject area using the experimental product for 21 days.

D28-Values of skin parameters in the subject area using the experimental product for 28 days.

Results and Analysis

Skin Elasticity R2

A higher R2 measurement value indicates better skin elasticity. As shown in Figure 1, Table 3.1, and Table 3.2, the average skin elasticity values collected from 33 subjects during the treatment period exhibited an upward trend compared to baseline values. A statistically significant difference ($P < 0.001$) was observed one week after treatment (D7), with an average improvement rate of 5.45% in skin elasticity R2. By D28, the average improvement rate

in skin elasticity R2 reached 12.73%. Based on this analysis, it can be concluded that the combined use of Monopolar Radio Frequency (RF) and recombinant type III collagen promotes the enhancement of skin elasticity (Table 3.1-3.3 and Figure 1).

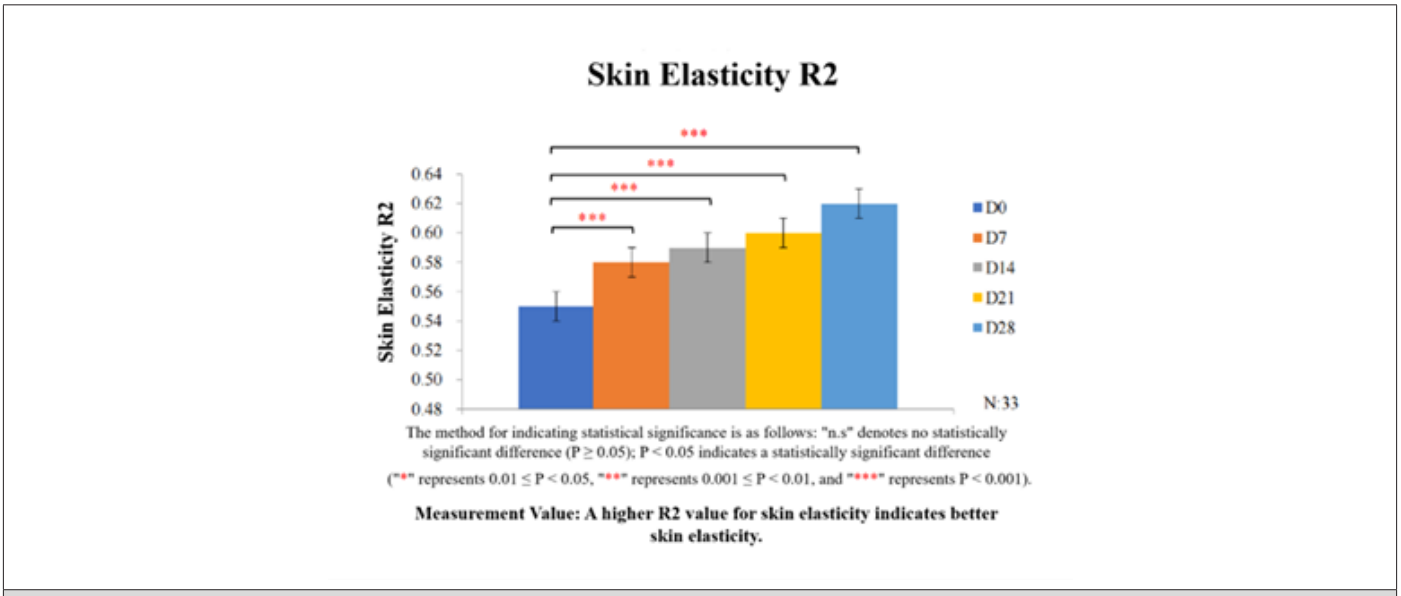

Figure 1: The Results of Skin Elasticity R2.

Skin Firmness F4

A lower measurement value for skin firmness F4 indicates tighter skin. As shown in Figure 2, Table 4.1, and Table 4.2, the average skin firmness values collected from 33 subjects during the treatment period exhibited a decreasing trend compared to baseline values. By D28, the highest average improvement rate in skin firmness F4 reached 18.59%. This analysis demonstrates that the combined application of monopolar Radio Frequency (RF) and recombinant type III collagen exerts a positive effect on enhancing skin firmness

(Table 4.1-4.3 and Figure 2).

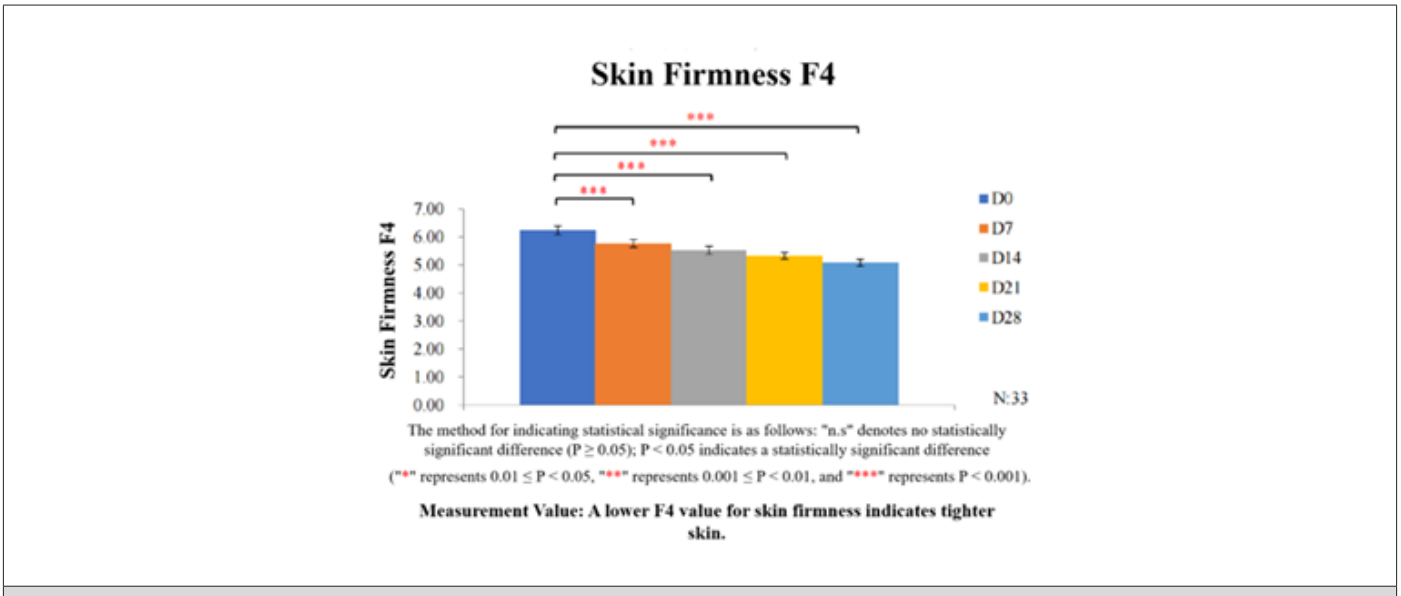

Figure 2: The Results of Skin Firmness F4.

Table 3.1: Test Results of Skin Elasticity R2 (Mean±Standard Error).

D0	D7	D14	D21	D28
0.55±0.01	0.58±0.01	0.59±0.01	0.60±0.01	0.62±0.01

Table 3.2: Improvement Rate of Skin Elasticity R2.

D7	D14	D21	D28
5.45%	7.27%	9.09%	12.73%

Table 3.3: Statistical Analysis Results of Skin Elasticity R2.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Wilcoxon signed rank test	0.0000	***
D14 vs. D0		Paired sample T-test	0.0000	***
D21 vs. D0		Paired sample T-test	0.0000	***
D28 vs. D0		Paired sample T-test	0.0000	***

Table 4.1: Test Results of Skin Firmness F4 (Mean±Standard Error).

D0	D7	D14	D21	D28
6.24±0.15	5.77±0.14	5.52±0.14	5.33±0.12	5.08±0.12

Table 4.2: Improvement Rate of Skin Firmness F4.

D7	D14	D21	D28
7.53%	11.54%	14.48%	18.59%

Table 4.3: Statistical Analysis Results of Skin Firmness F4.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Paired sample T-test	0	***
D14 vs. D0		Paired sample T-test	0	***
D21 vs. D0		Paired sample T-test	0	***
D28 vs. D0		Paired sample T-test	0	***

Stratum Corneum Hydration

As shown in Figure 3, Table 5.1, and Table 5.2, the average stratum corneum hydration values collected from 33 subjects during the treatment period exhibited an upward trend compared to baseline values. A significant increase in stratum corneum hydration was observed during the early treatment phase (D7 and D14, $P < 0.001$). Subsequently, the trend stabilized while maintaining a high level of moisture content, with an average improvement rate of 30.53% by D28. These findings indicate that the combined use of monopolar Radio Frequency (RF) and recombinant type III collagen promotes the enhancement of stratum corneum hydration (Table 5.1-5.3 and Figure 3).

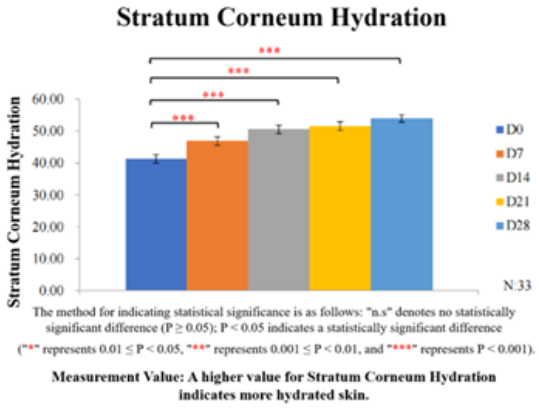

Figure 3: The Results of Stratum Corneum Hydration.

Table 5.1: Test Results of Stratum Corneum Hydration (Mean±Standard Error).

D0	D7	D14	D21	D28
41.37±1.32	**46.88±1.36**	**50.55±1.28**	**51.54±1.38**	**54.00±1.18**

Table 5.2: Improvement Rate of Stratum Corneum Hydration.

D7	D14	D21	D28
13.32%	22.19%	24.58%	30.53%

Table 5.3: Statistical Analysis Results of Stratum Corneum Hydration.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Paired sample T-test	0	***
D14 vs. D0		Paired sample T-test	0	***
D21 vs. D0		Paired sample T-test	0	***
D28 vs. D0		Paired sample T-test	0	***

Skin Color ITA° Value and Skin Color L* Value

Higher values of skin color ITA° and L* parameters indicate a lighter skin tone. As shown in Figure 4(a), Table 6.1, and Table 6.2, the average ITA° values of 33 subjects exhibited an upward trend compared to baseline measurements during the treatment period. A statistically significant improvement in ITA° values was observed at D7 and D21 ($P < 0.001$), with an average improvement rate of 20.59% by D28. Similarly, Figure 4(b), Table 7.1, and Table 7.2 demonstrate that the average L* values of the subjects also increased progressively, achieving an average improvement rate of 5.00% by D28. These findings indicate that the combined application of monopolar Radio Frequency (RF) and recombinant type III collagen exerts a positive effect on improving skin tone (Table 6.1-7.3 and Figure 4(a&b) & 4.1,4.2).

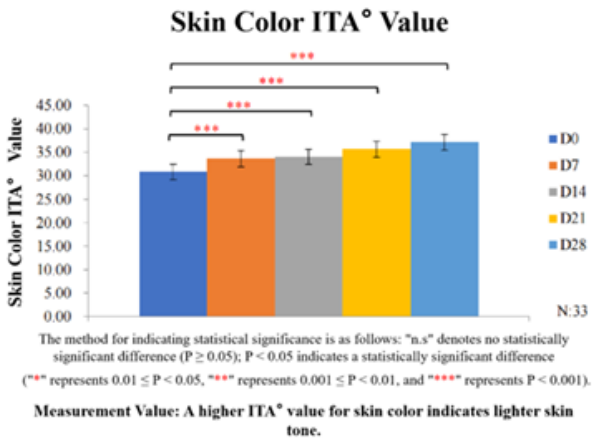

Figure 4(a): The Results of Skin Color ITA° Value.

Table 6.1: Test Results of Skin Colour ITA° Value (Mean±Standard Error).

D0	D7	D14	D21	D28
30.79±1.63	33.65±1.71	34.03±1.60	35.66±1.68	37.13±1.68

Table 6.2: Improvement Rate of Skin Colour ITA° Value.

D7	D14	D21	D28
9.29%	10.52%	15.82%	20.59%

Table 6.3: Statistical Analysis Results of Skin Colour ITA° Value.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Wilcoxon signed rank test	0	***
D14 vs. D0		Paired sample T-test	0	***
D21 vs. D0		Paired sample T-test	0	***
D28 vs. D0		Paired sample T-test	0	***

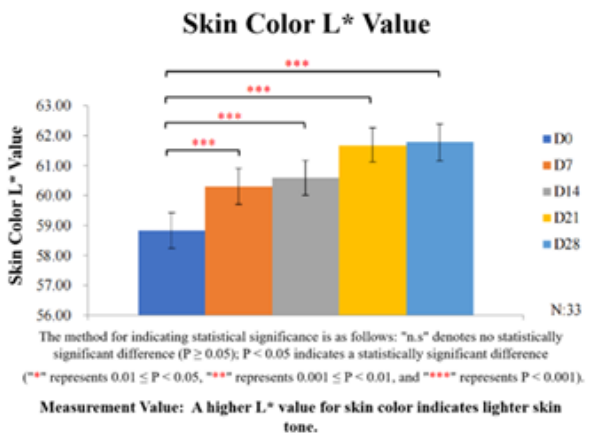

Figure 4(b): The Results of Skin Color L* Value.

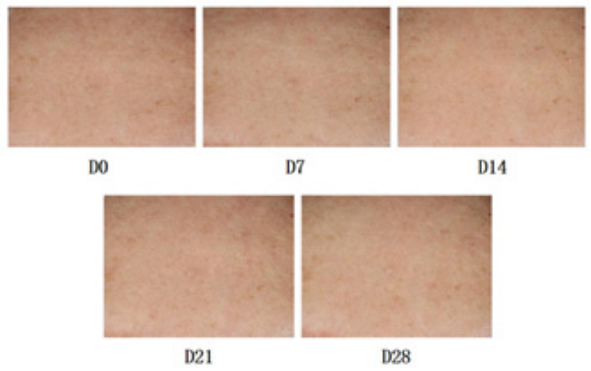

Figure 4.1: Effective Case 1 of Skin Tone Improvement by VISIA-CR(RD0002).

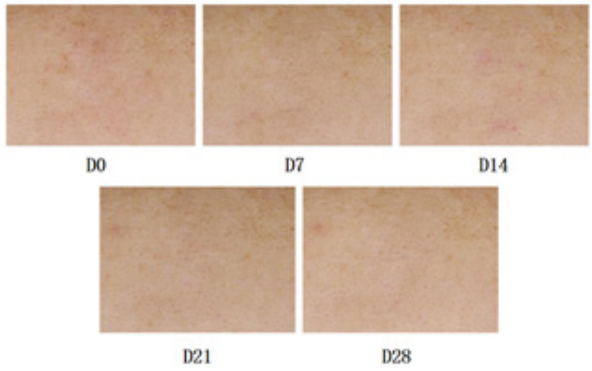

Figure 4.2: Effective Case 2 of Skin Tone Improvement by VISIA-CR(RD0028).

Table 7.1: Test Results of Skin Colour ITA° Value (Mean±Standard Error).

D0	D7	D14	D21	D28
58.83±0.59	60.31±0.60	60.59±0.58	61.69±0.57	61.77±0.61

Table 7.2: Improvement Rate of Skin Colour L* Value.

D7	D14	D21	D28
2.52%	2.99%	4.86%	5.00%

Table 7.3: Statistical Analysis Results of Skin Colour L* Value.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Wilcoxon signed rank test	0	***
D14 vs. D0		Wilcoxon signed rank test	0	***
D21 vs. D0		Wilcoxon signed rank test	0	***
D28 vs. D0		Wilcoxon signed rank test	0	***

Skin Gloss Parameter and VISIA-CR Skin Gloss Parameter

A higher value for the skin gloss parameter indicates better skin radiance. As shown in Figure 5(a), Table 8.1, and Table 8.2, the average skin gloss parameter values collected from 33 subjects during the treatment period exhibited an upward trend compared to baseline values, achieving an average improvement rate of 23.38% by D28. Similarly, Figure 5(b), Table 9.1, and Table 9.2 demonstrate that the average VISIA-CR skin gloss parameter values also progressively increased relative to baseline measurements, reaching an average improvement rate of 91.60% by D28. These results indicate that the combined application of monopolar radiofrequency (RF) and recombinant type III collagen effectively enhances skin gloss and promotes skin brightening (Table 8.1-9.3 and Figure 5(a&b) & 5.1,5.2).

Table 8.1: Test Results of Skin Gloss Parameter (Mean±Standard Error).

D0	D7	D14	D21	D28
7.70±0.25	8.68±0.20	8.83±0.23	9.21±0.21	9.50±0.16

Table 8.2: Improvement Rate of Skin Gloss Parameter.

D7	D14	D21	D28
12.73%	14.68%	19.61%	23.38%

Table 8.3: Statistical Analysis Results of Skin Gloss Parameter.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Paired sample T-test	0	***
D14 vs. D0		Wilcoxon signed rank test	0	***
D21 vs. D0		Paired sample T-test	0	***
D28 vs. D0		Paired sample T-test	0	***

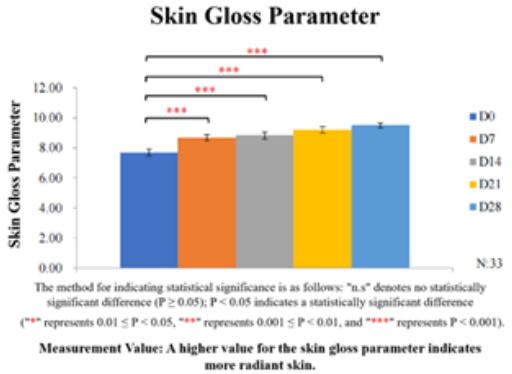

Figure 5(a): The Results of Skin Gloss Parameter.

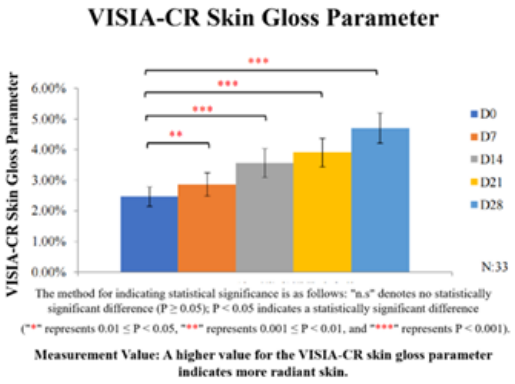

Figure 5(b): The Results of VISIA-CR Skin Gloss Parameter.

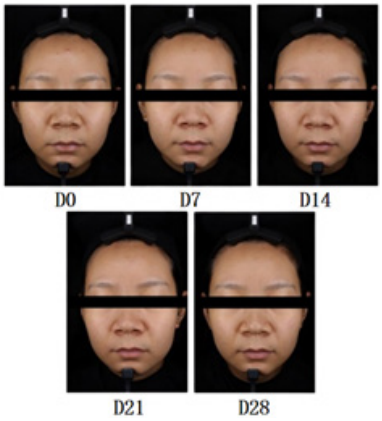

Figure 5.1: Effective Case 1 of Skin Gloss Improvement by VISIA-CR(RD0008).

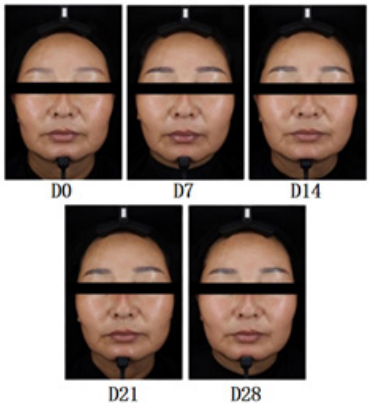

Figure 5.2: Effective Case 2 of Skin Gloss Improvement by VISIA-CR(RD0012).

Table 9.1: Test Results of VISIA-CR Skin Gloss Parameter (Mean±Standard Error).

D0	D7	D14	D21	D28
2.46%±0.32%	2.86%±0.38%	3.56%±0.47%	3.90%±0.47%	4.70%±0.49%

Table 9.2: Improvement Rate of VISIA-CR Skin Gloss Parameter.

D7	D14	D21	D28
16.26%	44.72%	58.54%	91.06%

Table 9.3: Statistical Analysis Results of VISIA-CR Skin Gloss Parameter.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Wilcoxon signed rank test	0.0014	**
D14 vs. D0		Wilcoxon signed rank test	0	***
D21 vs. D0		Wilcoxon signed rank test	0	***
D28 vs. D0		Paired sample T-test	0	***

VISIA-CR Under-Eye Wrinkle Area Percentage

As shown in Figure 6, Table 10.1, and Table 10.2, the average VISIA-CR under-eye wrinkle area percentage values collected from 33 subjects during the treatment period exhibited a decreasing trend compared to baseline values. Statistically significant differences were observed at D14 ($P < 0.01$), D21 ($P < 0.001$), and D28 ($P < 0.001$), indicating a progressive reduction in the under-eye wrinkle area as the number of treatment sessions increased. By D28, the average improvement rate in the VISIA-CR under-eye wrinkle area percentage reached 26.35%. These findings demonstrate that the combined application of monopolar radiofrequency (RF) and recombinant type III collagen exerts a positive effect on reducing the under-eye wrinkle area (Table 10.1-10.3 and Figure 6(a&b) & 6.1,6.2).

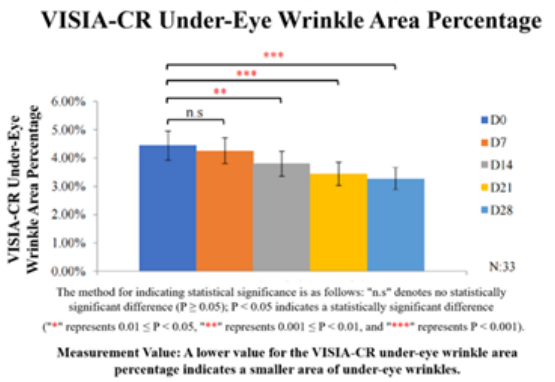

Figure 6: The Results of VISIA-CR Under-Eye Wrinkle Area Percentage.

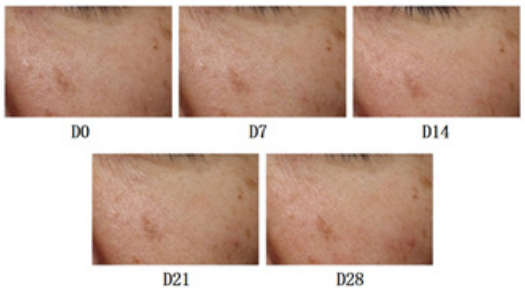

Figure 6.1: Effective Case 1 of Under-Eye Wrinkle Improvement by VISIA-CR(RD0002).

Figure 6.2: Effective Case 2 of Under-Eye Wrinkle Improvement by VISIA-CR(RD0024).

Table 10.1: Test Results of VISIA-CR Under-Eye Wrinkle Area Percentage (Mean±Standard Error).

D0	D7	D14	D21	D28
4.44%±0.51%	4.26%±0.45%	3.80%±0.44%	3.44%±0.41%	3.27%±0.39%

Table 10.2: Improvement Rate of VISIA-CR Under-Eye Wrinkle Area Percentage.

D7	D14	D21	D28
4.05%	14.41%	22.52%	26.35%

Table 10.3: Statistical Analysis Results of VISIA-CR Under-Eye Wrinkle Area Percentage.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Wilcoxon signed rank test	0.1503	n.s
D14 vs. D0		Paired sample T-test	0.0037	**
D21 vs. D0		Paired sample T-test	0.0000	***
D28 vs. D0		Paired sample T-test	0.0000	***

VISIA-CR Nasolabial Fold Area Percentage

As shown in Figure 7, Table 11.1, and Table 11.2, the average VISIA-CR nasolabial fold area percentage values collected from 33 subjects during the treatment period exhibited a progressive reduction compared to baseline values, with statistically significant differences ($P < 0.001$). This indicates that the nasolabial fold area gradually decreased as the number of treatment sessions increased. By D28, the average improvement rate in the VISIA-CR nasolabial fold area percentage reached 36.32%. These results demonstrate that the combined use of monopolar radiofrequency (RF) and recombinant type III collagen exerts a positive effect on reducing nasolabial folds (Table 11.1-11.3 and Figure 7(a&b) & 7.1,7.2).

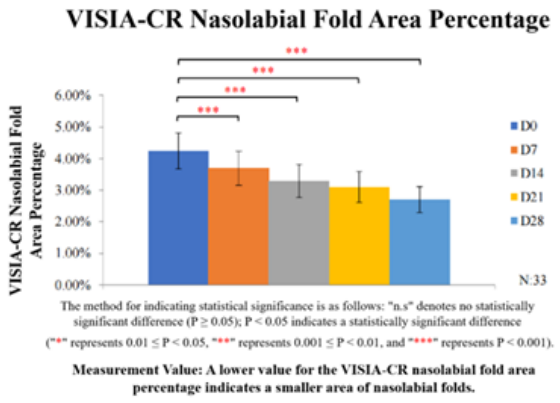

Figure 7: The Results of VISIA-CR Nasolabial Fold Area Percentage.

Table 11.1: Test Results of VISIA-CR Nasolabial Fold Area Percentage (Mean±Standard Error).

D0	D7	D14	D21	D28
4.24%±0.57%	3.69%±0.54%	3.29%±0.52%	3.10%±0.49%	2.70%±0.41%

Table 11.2: Improvement Rate of VISIA-CR Nasolabial Fold Area Percentage.

D7	D14	D21	D28
12.97%	22.41%	26.89%	36.32%

Table 11.3: Statistical Analysis Results of VISIA-CR Nasolabial Fold Area Percentage.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Wilcoxon signed rank test	0	***
D14 vs. D0		Wilcoxon signed rank test	0	***

D21 vs. D0	33	Wilcoxon signed rank test	0	***
D28 vs. D0		Wilcoxon signed rank test	0	***

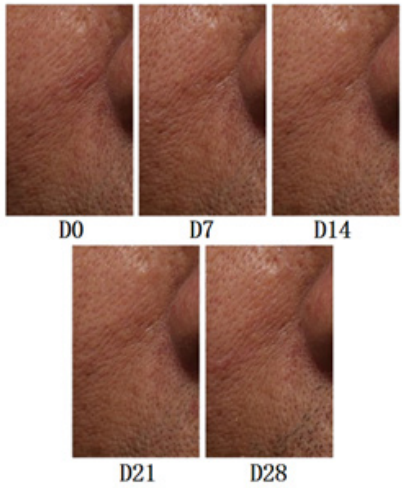

Figure 7.1: Effective Case 1 of Nasolabial Fold Improvement by VISIA-CR(RD0009).

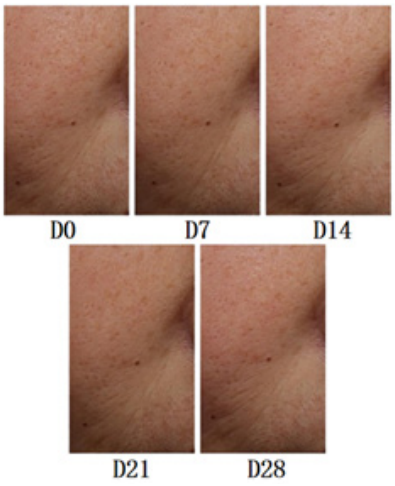

Figure 7.2: Effective Case 2 of Nasolabial Fold Improvement by VISIA-CR(RD0012).

VISIA-CR Canthal Wrinkle Area Percentage

As shown in Figure 8, Table 12.1, and Table 12.2, the average VISIA-CR canthal wrinkle area percentage values collected from 33 subjects during the treatment period exhibited a progressive reduction compared to baseline values, with statistically significant differences ($P < 0.001$). This indicates that the canthal wrinkle area gradually decreased as the number of treatment sessions increased. By D28, the average improvement rate in the VISIA-CR canthal wrinkle area percentage reached 41.64%. These results demonstrate that the combined use of monopolar radiofrequency (RF) and recombinant type III collagen exerts a positive effect on reducing canthal wrinkles (Table 12.1-12.3 and Figure 8(a&b) & 8.1,8.2).

Table 12.1: Test Results of VISIA-CR Canthal Wrinkle Area Percentage (Mean±Standard Error).

D0	D7	D14	D21	D28
5.86%±0.47%	4.91%±0.39%	4.52%±0.38%	3.93%±0.36%	3.42%±0.34%

Table 12.2: Improvement Rate of VISIA-CR Canthal Wrinkle Area Percentage.

D7	D14	D21	D28
16.21%	22.87%	32.94%	41.64%

Table 12.3: Statistical Analysis Results of VISIA-CR Canthal Wrinkle Area Percentage.

Comparison Time Points	N	Statistical Methods	P-value	Significance
D7 vs. D0	33	Wilcoxon signed rank test	0	***
D14 vs. D0		Wilcoxon signed rank test	0	***
D21 vs. D0		Wilcoxon signed rank test	0	***
D28 vs. D0		Wilcoxon signed rank test	0	***

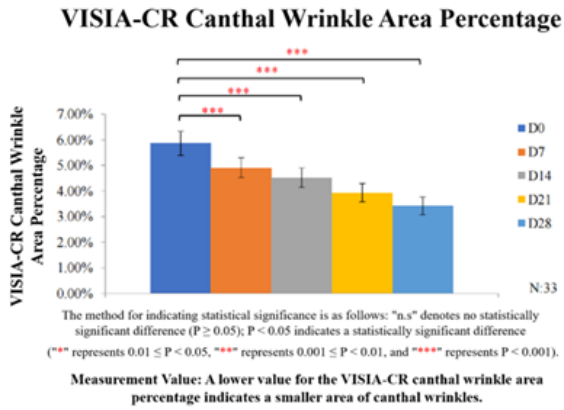

Figure 8: The Results of VISIA-CR Canthal Wrinkle Area Percentage.

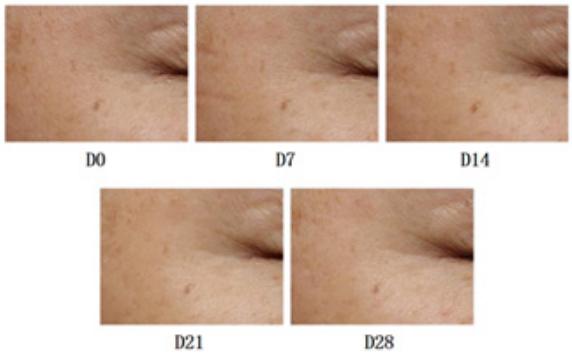

Figure 8.1: Effective Case 1 of Canthal Wrinkle Improvement by VISIA-CR(RD0002).

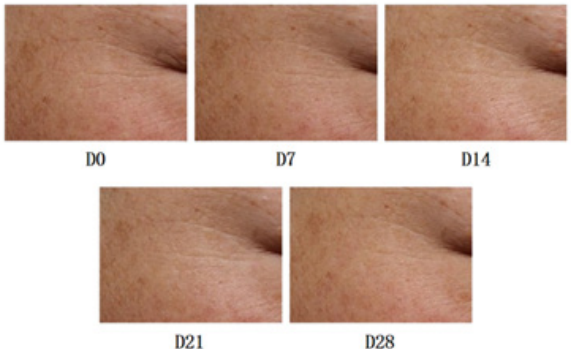

Figure 8.2: Effective Case 2 of Canthal Wrinkle Improvement by VISIA-CR(RD0029).

Subjective Evaluation Questionnaire Statistical Results

(Table 13).

Table 13: Subjective Evaluation Questionnaire Statistical Results.

Evaluation indicators	Identification rate（≥4）	P-value	Significance
After use, the forehead wrinkles have been improved	100.00%	0	***
After use, the nasolabial folds have been improved	100.00%	0	***
After use, the crow's feet have been improved	100.00%	0	***
After use, it can brighten facial skin tone	100.00%	0	***
After use, the skin's hydration have been improved	100.00%	0	***
After use, the elasticity of facial skin has been improved	100.00%	0	***
After use, the tightness of facial skin has been improved	100.00%	0	***
After use, the facial skin becomes more radiant	96.97%	0	***
This treatment is mild and non-irritating	100.00%	0	***

Safety Evaluation

Throughout the treatment period, all 33 subjects were monitored via interviews and clinical examinations for any cutaneous or systemic adverse reactions. Parameters including the manifestation, time of occurrence, management measures, and outcomes of adverse events (AEs) were recorded, and the causality between AEs and the investigational product was evaluated. No cutaneous adverse reactions were observed in any subject during the entire treatment course (Table 14).

Table 14: Safety Evaluation Results.

Reaction	Grade	Number of Cases
No reaction	0	33 cases
Mild erythema	1	0 cases
Erythema, infiltration, papules	2	0 cases
Erythema, edema, papules, vesicles	3	0 cases
Erythema, edema, bullae	4	0 cases

Conclusion

The results demonstrate that after four consecutive applications (D28) of monopolar radiofrequency (RF) combined with recombinant type III collagen on facial skin, subjects exhibited significant improvements across multiple parameters compared to baseline (D0):

I. Skin elasticity R2 improvement rate: 12.73%;

II. Skin firmness F4 improvement rate: 18.59%;

III. Stratum corneum moisture content improvement rate: 30.53%;

IV. Skin color ITA° value improvement rate: 20.59%;

V. Skin color L* value improvement rate: 5.00%;

VI. Skin gloss parameter improvement rate: 23.38%;

VII. VISIA-CR skin gloss parameter improvement rate: 91.06%;

VIII. VISIA-CR nasolabial fold area percentage improvement rate: 36.32%;

IX. VISIA-CR canthal wrinkle area percentage improvement rate: 41.64%.

Statistically significant improvements ($P < 0.01$) were observed for all parameters by D28. Notably, the VISIA-CR under-eye wrinkle area percentage showed a 26.35% improvement at D28, with significant differences emerging after D14 ($P < 0.01$). These findings indicate that the combined use of monopolar RF and recombinant type III collagen effectively enhances anti-wrinkle efficacy, hydration, skin firmness, and brightness. Importantly, no cutaneous adverse reactions were observed in any of the 33 subjects throughout the study. Thus, this regimen demonstrates a clinically meaningful and safe approach to mitigating facial skin aging.

Discussion

Monopolar Radio Frequency (RF) is widely regarded as the gold standard for non-invasive treatment of skin laxity [19]. Its efficacy stems from deep tissue penetration, enabling bypass of the epidermal basal melanocyte barrier to deliver targeted heating to dermal collagen fibers. This thermal energy induces immediate collagen contraction and promotes neogenesis/remodelling, achieving sustained skin tightening and wrinkle reduction [12,20].

An in vivo study by Dong Hye Suh et al. [21] involving 11 subjects demonstrated that monopolar RF treatment significantly increased collagen fiber density within the dermis, as evidenced by data collected at 2 and 6 months post-treatment compared to baseline. Furthermore, monopolar RF generates heat extending to the subcutaneous fat layer, inducing thermally induced contraction of fibrous septa. This mechanism contributes to skin tightening along the perpendicular axis, enhancing overall contour [22].

Recombinant type III collagen, frequently incorporated into cosmetic formulations to leverage its anti-aging, wound-healing, and moisturizing properties [8], has gained increasing prominence in the skincare market due to its high safety profile and superior biocompatibility. Studies indicate that after 8 weeks of integration with the Extracellular Matrix (ECM), recombinant type III collagen significantly stimulates the regeneration of type I and type III collagen within the dermis while facilitating ECM remodelling [23]. Research by Fan Ting et al. [24] demonstrated that recombinant type III collagen enhances collagen synthesis, inhibits collagen degradation, strengthens the skin barrier, and improves hydration, thereby exhibiting remarkable anti-wrinkle efficacy.

It is noteworthy that monopolar Radio Frequency (RF), as a non-invasive skin-tightening modality, has not been associated with reports of severe adverse reactions. Clinically observed complications primarily include transient side effects such as burning sensations, erythema, superficial second-degree burns, and epidermal dehydration due to elevated skin temperatures [25].

In this study, the combined application of monopolar RF and recombinant type III collagen on the facial skin of 33 subjects demonstrated a progressive increase in stratum corneum moisture content at D7, D14, D21, and D28; this increase significantly exceeded baseline (D0) measurements ($P < 0.001$), with no adverse reactions observed. These findings confirm that recombinant type III collagen effectively mitigates RF-induced epidermal dehydration while enhancing hydration, repair, and barrier restoration.

In summary, this study investigated the anti-aging efficacy of a combined regimen utilizing monopolar Radiofrequency (RF) and recombinant type III collagen on facial skin. Post-treatment evaluations revealed significant improvements in skin biomechanical properties, including enhanced firmness, elasticity, and stratum corneum moisture content. Additionally, quantitative assessments demonstrated reductions in under-eye wrinkles, canthal wrinkles, and nasolabial folds, accompanied by improvements in skin tone homogeneity and luminosity.

These findings validate the therapeutic potential of the combined modality for mitigating mild-to-moderate facial skin laxity and attenuating wrinkle severity, with no treatment-related adverse events reported. However, several limitations warrant consideration, such as the small sample size and short observation period. To strengthen the validity of these preliminary results, future investigations should prioritize large-scale randomized controlled trials with extended follow-up to evaluate treatment durability and long-term safety profiles. Furthermore, elucidating the interplay between RF-induced collagen remodelling and exogenous collagen supplementation, and exploring responses specific to diverse Fitzpatrick skin types and photodamage severity, represent critical avenues for advancing clinical translation.

Author Contributions

Conceptualization, Y.J, D.H-M ; methodology, Y.J, D.H.M ; validation,Y.J, D.H.M ; data curation, Y.M-Y,L.J-L,L.Q-Y,Z.J,F.W-S and W.C-Y; writing, Y.J, W.S-T,Y.M-Y and W.C-Y; visualization, Y.J, W.S-T,L.J-L,Y.M-Y and W.C-Y; All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement: This study was conducted in compliance with the Declaration of Helsinki and its subsequent amendments. All volunteers participating in this project signed the informed consent forms.

Informed Consent Statement: Written informed consent has been obtained from the volunteers to publish this paper.

Data Availability Statement: Data are available upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. M Gniadecka, O F Nielsen, S Wessel, M Heidenheim, D H Christensen, et al. (1998) Water and protein structure in photoaged and chronically aged skin. J Invest Dermatol 111(6): 1129-1133.

2. Anna Sorushanova, Luis M Delgado, Zhuning Wu, Naledi Shologu, Aniket Kshirsagar, et al. (2019) The Collagen Suprafamily: From Biosynthesis to Advanced Biomaterial Development. Adv Mater 31(1): 1801651.

3. Rong YH, Zhang GA, Wang C, Ning FG (2008) [Quantification of type I and III collagen content in normal human skin in different age groups]. Zhonghua Shao Shang Za Zhi 24(1): 51-53.

4. Kuivaniemi H,Tromp G (2019) Type III collagen (COL3A1): gene and protein structure, tissue distribution, and associated diseases. Gene 707:151-171.

5. Sam Shuster, Martin M Black, Eva Mcvitie (1975) The influence of age and sex on skin thickness, skin collagen and density. Br J Dermatol 93(6): 639-643.

6. Rong yanhua, Zhang guoan, Wang cheng (2008) Quantification of type I and III collagen content in normal human skin in different age groups. Chinese Journal of Burns and Wounds 24(1): 51-53.

7. Zhou Xiaohui, Chang Yanan, HE Xiaoliang (2017) Structure, function and latest research development of collagen of type II. Journal of Hebei University of Science and Technology 38(2): 202-208.

8. Zhang Ya, Sun Xin, Wang Rui Yan (2024) Review of the Application of Recombinant Type III Collagen in Skincare and Pharmaceutical Devices. Shanghai Light Industry (4): 133-135.

9. Xue Chun yu, Wang Yu chong (2021) Review of facial rejuvenation methods. Chinese Journal of Aesthetic and Plastic Surgery 32(2): 65-68.

10. Lang Xun, Li Li, Zhong JiaXuan (2023) New progress in the application of facial rejuvenation radiofrequency technology in China. Chinese Journal of Aesthetic and Plastic Surgery 34(6): 345-349.

11. Galen H Fisher, Laurie G Jacobson, Leonard J Bernstein, Karen H Kim, Roy G Geronemus (2005) Nonablative radiofrequency treatment of facial laxity. Dermatol Surg (9 Pt.2): 1237-1241.

12. Yang RongYa, Yin Yue (2021) Experts consensus on the application of medical radiofrequency in cosmetic dermatology and treatment. Chinese Journal of Aesthetic and Plastic Surgery 14(4): 193-197.

13. Flament F, Bazin R (2010) Skin Aging Atlas Volume 2: Asian Type.

14. (2023) Evaluation of cosmetics for sensitive skin: T/GDCA 029-2023.

15. Liu Wei-Yi; ZHOU Lin; Zhao Hua (2021) Efficacy evaluation of cosmetics (X III) Consumer use testing. China Surfactant Detergent & Cosmetics 51(6): 485-490.

16. (2022) Seven Efficacy Test Methods for Anti-Wrinkle, Firming, Moisturizing, Oil Control, Repairing, Nourishing and Soothing of Cosmetics: T/CAB 0152-2022.

17. (2021) Method for Assessment of Cosmetics Firmness Efficacy: T/TDCA 003-2021.

18. Alster TS, Lupton JR (2007) Nonablative cutaneous remodeling using radiofrequency devices. Clin Dermatol 25(5): 487-491.

19. Abraham MT, Mashkevich G (2007) Monopolar radiofrequency skin tightening. Facial Plast Surg Clin North Am 15(2): 169-177.

20. LI Jun, LI Qian, Gao Yan li (2013) Observation and clinical application of monopolar radiofrequency for the facial rejuvenation. Chinese Journal of Aesthetic Medicine 22(2): 274-277.

21. Dong Hye Suh, Hye Jin Ahn, Jong Kil Seo, Sang Jun Lee, Min Kyung Shin, et al. (2020) Monopolar radiofrequency treatment for facial laxity: Histometric analysis. J Cosmet Dermatol 19(9): 2317-2324.

22. Melissa A Bogle, Nathan Ubelhoer, Robert A Weiss, Flor Mayoral, Michael S Kaminer (2007) Evaluation of the multiple pass, low fluence algorithm for radiofrequency tightening of the lower face. Lasers Surg Med 39(3): 210-217.

23. Jing Wang, He Qiu, Yang Xu, Yongli Gao, Peijie Tan, et al. (2021) The biological effect of recombinant humanized collagen on damaged skin induced by UV-photoaging: An in vivo study. Bioact Mater 11: 154-165.

24. Fan Ting, Zhao Jian Fen, Chang YeJun (2022) Effect of recombinant humanized type III collagen on expression of skin functional related genes. China Surfactant Detergent & Cosmetics 52(12): 1326-1332.

25. CHEN Ying Tao, Huang Lv Ping, Feng Yong Qiang (2021) Complications analysis of monopolar radiofrequency therapy for facial aging. Chinese Journal of Medical Aesthetics and Cosmetology 27(6): 551-552.