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Safety and Efficacy Evaluation of Composite Collagen in Human Infraorbital Anti-aging and Nude Mouse Skin Photoaging

Tao Wang[1,2] · Aawrish Khan[1,2] · Lunli Gong[1,2] · Kim Hong[1,2] · Lili Qi[1,2] · Haiyan Cui[1,2]

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Abstract

Background Collagen is currently a widely used injectable filler material. Due to the complexity and specificity of the infraorbital structure and function, it undergoes various aging changes earlier in the facial aging process. Additionally, continuous exposure to Ultraviolet (UV) leads to photoaging of the skin. In this study, we utilized type I and type III composite collagen as a filler material for injecting the infraorbital region of the human face and the dorsal region of a nude mouse skin photoaging model to assess its effectiveness and safety.

Objective To assess the effectiveness of type I and type III composite collagen in treating age-related changes in the human infraorbital area, as well as in a nude mouse model of skin aging.

Methods A total of 36 patients with infraorbital aging were enrolled to receive type I and type III composite collagen injections. The improvement of infraorbital aging was assessed at pre-injection, immediate post-injection, 1 week, 4 weeks, and 12 weeks. Additionally, nude mice photodamaged models were prepared and collagen injections were administered to treat photodamaged skin. The therapeutic efficacy was assessed by gross view, histological staining, gene expression analysis, and ELISA assay.

Results Type I and Type III composite collagen injection can immediately fill depressed areas. However, one week after the injection, the collagen dehydrates and contracts, causing the material to be absorbed by the tissues and resulting in a slight regression of the filling effect. After one month, some of the collagen has degraded, with most of it degrading after three months. Additionally, in treating photoaging, type I and type III composite collagen has a more pronounced therapeutic effect on photoaging and demonstrates better results in collagen regeneration, inflammation reduction, and melanin control.

Conclusion Type I and Type III composite collagen demonstrates superior efficacy and safety in addressing infraorbital aging and photoaging in nude mice skin, making it a more favorable choice as an injection material.

Level of Evidence II This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors www.springer.com/00266.

Keywords Minimally invasive cosmetic · Composite collagen · Skin photoaging

Introduction

With the advancement of social aesthetics and the widespread promotion of aesthetic education, there is a growing demand for facial beautification and youthfulness among people. In clinical practice, non-surgical procedures such as collagen and hyaluronic acid injections, as well as photoelectric treatments like intense pulsed light, have seen a significant increase in frequency over the years [1, 2]. This is due to their minimally invasive nature, short

Tao Wang and Aawrish Khan have contributed equally to this work.

✉ Haiyan Cui
u2beauty1@sina.com

[1] Department of Plastic and Cosmetic Surgery, Tongji Hospital, School of Medicine, Tongji University, Shanghai 200092, People's Republic of China

[2] Institute of Aesthetic Plastic Surgery and Medicine, School of Medicine, Tongji University, Shanghai 200092, People's Republic of China

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recovery period, and the ability for patients to resume normal activities without prolonged downtime [3]. As a result, these non-invasive treatments have far surpassed surgical procedures in terms of frequency.

In the field of facial anti-aging, we have developed a design method for "Future 未来" aesthetic facial anti-aging that is based on oriental culture and the anatomical characteristics of oriental people [4]. This method has been refined over 30 years of our clinical experience and offers a simple way to express professional medical cosmetology knowledge. It is particularly useful for beginners in medical cosmetology teaching, as it allows them to quickly grasp the essentials of facial medical cosmetology treatment. One important aspect of this "Future 未来" aesthetic system is orbital decay resistance, which contributes to a youthful appearance in the face. Aging can be caused by autologous factors leading to local subcutaneous tissue loss and skin ligament relaxation, as well as external UV radiation resulting in skin photoaging characterized by collagen loss, increased wrinkles, pigmentation issues, and more [5, 6].

Cosmetic injection technology has proven to be an effective treatment method for addressing these signs of aging. Currently, there are three main categories of injection materials commonly used in clinical applications: natural biological materials (such as collagen and hyaluronic acid) [7], autologous transplantation materials (including autologous fat granules and fibroblasts) [8], and artificial synthetic materials (like poly-L-lactic acid) [9]. Among these options, although collagen products have disadvantages such as sensitization and short maintenance time, it has become popular due to their low risk of embolism, low antigenicity, absorbable degradation properties, and filling capabilities, among other features. A variety of collagen types have been developed including those of animal or human origin such as bovine or porcine collagen; patient's own skin; cadaveric skin; and collagen cultured from fibroblasts [3, 10, 11].

The complex collagen is gaining increasing attention due to its inclusion of both type I and type III collagen, which are essential components in human skin. Type I collagen constitutes 80–85% of the skin and forms a thick, tightly arranged bundle structure with tensile strength that exceeds that of steel wire. It provides crucial support and structure for the skin, and loss of type I collagen can lead to facial wrinkles and depression. On the other hand, type III collagen makes up 10–15% of the skin and exists in the form of a loose filament network, mainly present in the dermal-epidermal junction to provide elasticity and stress resistance to the skin [12].

In this study, we recruited 36 patients with infraorbital aging and administered composite collagen injections. Furthermore, we established a nude mouse photoaging model to further evaluate the effectiveness of collagen at an animal level. This study aims to assess the safety and efficacy of collagen in both human and nude mouse models, providing evidence for its anti-aging properties.

Methods

Clinical Observational Assessment

Inclusion and Exclusion Criteria

Inclusion criteria: Healthy women between the ages of 20 and 60 who have been evaluated as suitable candidates for periorbital aging. Exclusion criteria: Individuals who test positive for collagen allergies (such as egg, milk, or other high protein allergies), those with a history of allergies, individuals with immune disorders, individuals who have undergone facial plastic surgery or received injectable fillers, patients with serious medical conditions, pregnant women, and any other participants deemed by the doctor to potentially impact the effectiveness of the injections after evaluation.

Injectable Products

The source was type I and type III composite bovine skin collagen (1.0 ml/count, Fillderm, China) with a collagen content of 3.5% (35 mg/ml, including 85% type I collagen and 15% type III collagen) and a lidocaine content of 0.3%.

Study Design

This retrospective study systematically analyzed the clinical data of 36 patients treated for facial periorbital aging in the infraorbital region between May 2023 and July 2023 at our hospital. Institutional ethics committee approval was obtained before the study commenced, and written informed consent was obtained from all participants prior to treatment initiation. Permission was also granted to use the clinical data for further studies. The feasibility of this injection method, as well as the efficacy and safety of type I and type III composite collagen fillers for improving infraorbital aging, were evaluated through before-and-after controls based on the results of the photo collection.

Treatment Method

Before injection, the face should be thoroughly cleansed. The surface anesthetic ointment should then be applied for 40 min, during which time any signs of redness, itching, or other allergic reactions should be carefully observed. After removing the surface anesthesia ointment and disinfecting

the entire facial area with iodophor solution, collagen injection can be performed in the infraorbital region. Sharp 30G cannulas and blunt 27G cannulas are used for filling the periosteal layer.

Sharp 30G cannulas and blunt 27G cannulas are used for filling the periosteal. Sector injections are performed at 4–5 points per line with a dosage of 0.01–0.02 mL per point followed by pressure application after each injection. The total dose of injection was 2ml on both sides. After injection, use the medical mask for 15 minutes.

Effect Evaluation

Facial photographs were taken before injection, immediately after injection, at 1 week, 4 weeks, and 12 weeks to assess the treatment effect in the infraorbital region.

Evaluation of Photoaging Model in Nude Mice

Model Preparation

The animal study was approved by the Laboratory Animal Ethics Committee of Hospital. Twenty healthy male nude mice at 6 weeks of age, weighing 12–16 g, were used for the experiment. The nude mice were acclimatized and fed in laboratory conditions for 1 week before the formal start of the experiment. To establish and maintain the UV-induced photoaging skin model, nude mice skin was irradiated with UVA and UVB lamps at a height of 30 cm. The minimum erythemal dose (MED) is defined as the amount of UV radiation that can cause observable erythema to the naked eye. The nude mice underwent three phases of UV irradiation: In the first phase, which lasted for 2 weeks, they received 1 MED every other day. The second phase lasted for another 2 weeks with an increased dose of 2 MEDs every other day. In the fifth to eighth week, they were irradiated three times every other day. The entire irradiation cycle lasted for 8 weeks, resulting in cumulative doses of UVA and UVB at 105.84 J/cm2 and 24.57 J/cm2, respectively. Irradiations were stopped when blisters, ulcers, or vesicles appeared on their backs.

Subsequently, the nude mice were randomly divided into two groups: an untreated group and a treated group; each consisting of ten animals. The untreated group was given a normal diet without any treatment while in the treatment group, collagen injections (0.5 mL) were administered into their exposed back skin every two weeks using a sharp cannula (30 G). After eight weeks, samples from their skin were collected to evaluate treatment effects through visual assessment as well as histological staining, PCR detection, and ELISA quantitative assay to assess changes on a molecular level.

Histological Observation

At week 9, all nude mice were euthanized with an overdose of ketamine. Following euthanasia, the skin and subcutaneous tissues in the treatment area were promptly excised from the nude mice and cut into small pieces approximately 4.0 mm*20.0 mm in size, which were then rinsed with pre-cooled saline. The tissues were fixed in 4% paraformaldehyde for a minimum of 24 h before undergoing dehydration and other necessary treatments. Subsequently, the tissues were embedded in paraffin and sectioned into 5 μm slices.

Hematoxylin and Eosin (HE) staining was utilized to observe cell and stromal distribution as well as assess epidermal and dermal thickness; Masson's trichrome staining was employed to visualize collagen fiber arrangement; Sirius red staining was used to differentiate between different types of collagens within the skin as well as evaluate regeneration levels; β-Galactosidase (β-Gal) immunofluorescence staining was performed to analyze collagen fibers, cellular senescence within the tissues was assessed using β-Gal immunofluorescence staining; inflammation levels were evaluated through IL-6 and TNF-α immunofluorescence staining. Meanwhile, DAPI staining was used to detect nuclei.

RNA Isolation and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

RNA from tissues was extracted and isolated using TRIZOL reagent (Ambion, Austin, TX, USA) according to the manufacturer's instructions. The extracted RNA was reverse transcribed by iScript™ cDNA Synthesis Kit (BioRad, Hercules, CA, USA) to obtain the cDNA template for RT-qPCR amplification. Primer sequences for Elastic, MMP3, MMP9, TRP-1, and TYR genes were purchased from Beyotime Biotech Inc., and the GAPDH gene mRNA level was used as an endogenous control. Each sample was analyzed three times.

ELISA Quantification

The skin tissues were weighed, then added with PBS (pH=7.4), homogenized using an ultrasonic crusher, and centrifuged at 4 °C and 2500 rpm for 10 min. The absorbance values of each well were measured at 450 nm according to the instructions provided by the manufacturer of the ELISA kits. Subsequently, the absorbance values of each well were calculated based on the standard curve and protein concentration. Hydroxyproline (Hyp), superoxide dismutase (SOD), malondialdehyde (MDA), and α-melanocyte stimulating hormone (α-MSH) levels were

determined from the standard curve and protein concentration in each tissue.

Statistical Analysis

The statistical difference between the untreated group and the treated group was evaluated by one-way analysis of variance (ANOVA). All data were expressed as mean ± standard deviation and were considered statistically significant at $P \leq 0.05$. Data were analyzed using GraphPad Prism 8.0.2 software.

Results

Clinical Study

A 24-year-old healthy woman was enrolled in this study and assessed by "Future未来" Aesthetics for significant infraorbital aging, which was determined to be mild (Fig. 1). Collagen, applied to the infraorbital region in a volume of 2 mL, proved to be an effective filler and wrinkle reducer. Immediately after injection, there was noticeable filling of the depressed area and improved wrinkles (Fig. 2B). One week post-injection, the collagen dehydrated and contracted, with the water in the material being absorbed by the tissues. This resulted in a slight reduction in filling effect without any obvious bruising or allergic reactions (Fig. 2C). By one month post-injection, some of the collagen had degraded and there was a decrease in wrinkle improvement (Fig. 2D). After three months post-injection, degradation continued to increase leading to a further drop in depression improvement. However, overall skin quality improved and wrinkle improvement met rejuvenation standards (Fig. 2E). These findings suggest that degraded amino acid products can continue to provide nutrient circulation for tissues while also inducing cell proliferation and differentiation for synthesizing newborn collagen. The collagen fiber scaffolding network provides attachment points for the growth of newborn collagen resulting in better skin rejuvenation [13].

Evaluation of Photoaging Treatment Effect in Nude Mice

The nude mice were irradiated with UV light according to the established procedure. The general observation revealed that the skin of the nude mice prior to model preparation was smooth, tender, and exhibited normal color and elasticity (Fig. 3A). In contrast, the skin of the nude mice after successful preparation of the photoaging model showed roughness, flaccidity, hyperpigmentation, and debris (Fig. 3B). HE staining and Masson staining of the nude mouse skin demonstrated that prior to model preparation (Fig. 3C, D), the epidermal layer of dorsal skin was clear with neatly arranged cells and rich fibrous tissues in the dermis. The collagen fibers displayed uniform thickness and distribution without obvious fracture or disordered arrangement. Following the successful preparation of the photoaging model (Fig. 3E, F), there was a noticeable thickening of the epidermal layer along with loosely arranged collagen fibers. Additionally, more inflammatory cells infiltrated throughout the dermis compared to pre-preparation conditions. These findings indicate a typical appearance of photoaging, confirming successful model preparation.

Evaluation of the Effect of Collagen in the Treatment of Skin Photoaging

As depicted in Fig. 4, following collagen treatment, the treated group exhibited a more pronounced improvement in overall skin condition. In the untreated group (Fig. 4A), there was some improvement in skin aging compared to the model preparation (Fig. 3B), but the skin still appeared rough, with some debris and erythema, as well as more noticeable hyperpigmentation. Conversely, in the treatment group (Fig. 4B), photoaging was reduced and the skin appeared significantly smoother and finer, with no apparent erythema or hyperpigmentation.

In Fig. 4C and D, it is evident that the untreated group still displayed obvious signs of photoaging: thickened and irregularly arranged skin with noticeable vacuolated changes. The collagen fibers were also found to be disordered, exhibiting fracture and fragmentation. In contrast, in the treated group (Fig. 4E, F), nude mice's skin showed clear layering and structural integrity after collagen injection. Furthermore, there were no other lesions evident post-treatment. Additionally, epidermal thickness decreased compared to that of the untreated group but approached normal levels for rats; vacuolar patterns improved; and collagen fibers exhibited a certain degree of fragmentation and breakage.

Evaluation of Sirius Red, Cellular Senescence, and Inflammation Indices in Collagen Treatment of Photoaging

Sirius red lining stains readily bind to basic groups in collagen molecules. By using polarized light microscopy, it is possible to differentiate between type I and type III collagen fibers; type I collagen fibers appear orange or red, while type III collagen fibers appear green (Fig. 5A, B). The distribution of both red and green colors in the untreated group (Fig. 5A) was sparser than in normal nude

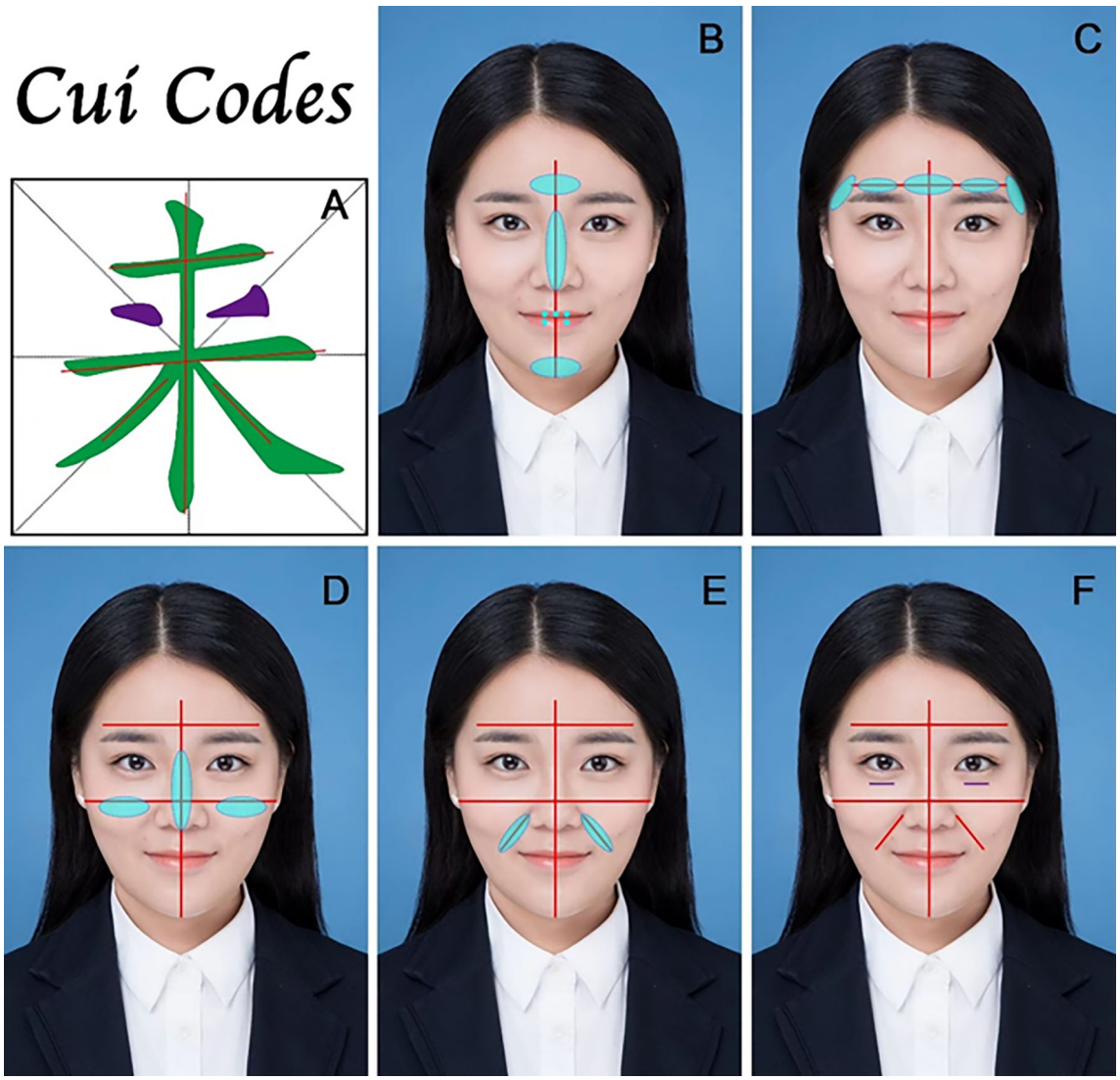

Fig. 1 "Future 未来" based on Chinese calligraphy. **A** The 2 Chinese characters "未来" which means "future" in English. The concept encompasses the systematic overall design for the art of facial injection in Asians. **B** The middle line of the face passes through the forehead, the glabella complex, the nose, the lips, and the chin. **C** The first horizontal line passes the arch of the temper region, the eyebrow, and the glabella complex. **D** The second horizontal line passes through the cheeks or the so-called apple muscle. **E** The other 2 oblique lines run along the nasolabial folds. **F** Finally, 2 nasojugular folds or tear troughs, are added. This figure is referenced in our previously published article (https://doi.org/10.1093/asjof/ojaa053)

mouse skin, suggesting that UV radiation leads to dermal damage and loss of COL I as well as COL III. Combining the images, it can be seen that the total collagen content and density in the treated group (Fig. 5B) were higher than those in the untreated group. There was a significant increase in COL I, while COL III did not change significantly. This may be due to the fact that collagen contains 85% type I collagen and 15% type III collagen, which replenishes the skin with collagen.

β-Gal is a commonly used marker of cellular senescence with increased activity during cellular senescence. By detecting the level of β-Gal in cells, one can assess the degree of cellular senescence. The untreated group exhibited a higher green fluorescence expression (Fig. 5C), confirming significant cell senescence. In contrast, the green fluorescence expression in the treated group (Fig. 5D) was significantly reduced, possibly due to the diverse and biomimetic ratio of collagen present in this group allowing for nourishment and repair of nude mice skin.

Skin damage caused by UV rays is primarily mediated directly or indirectly by inflammation and oxidative stress [14–16]. Following UV irradiation, immune disorders may occur in the skin, thereby activating inflammation. Subsequently, cells in the epidermis and dermis will produce a series of inflammatory factors, including IL-1, IL-6, IL-10,

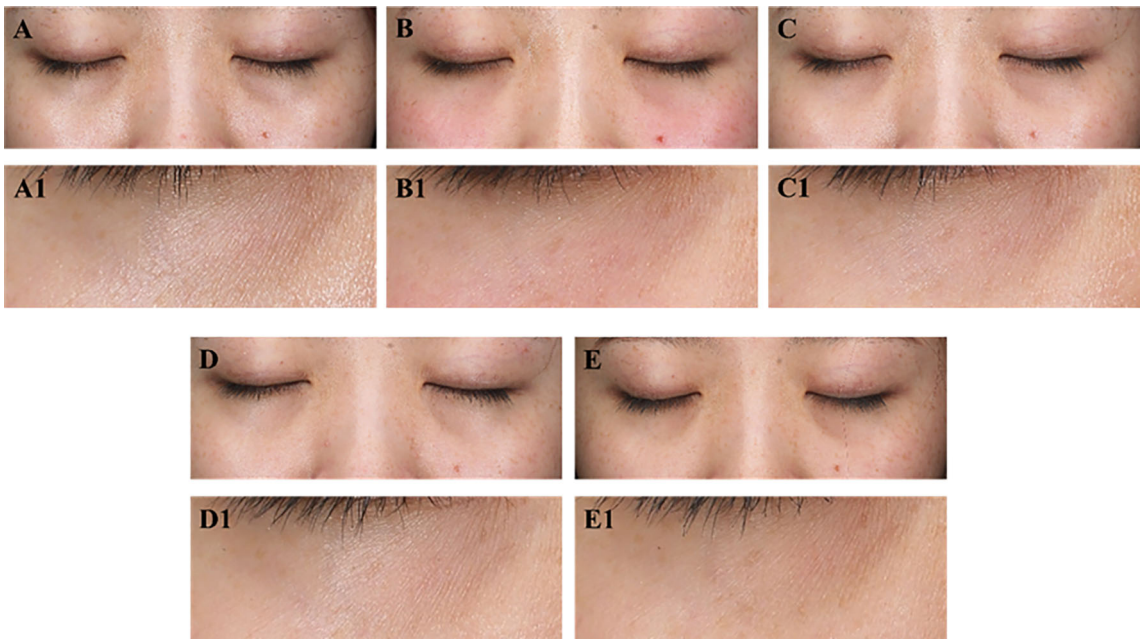

Fig. 2 A 24-year-old woman received collagen injections. **A** Before injection. **B** Immediately after injection. **C** One week after collagen injection. **D** One month after collagen injection. **E** Three months after collagen injection. Where **A1**–**E1** represent localized magnified images of the right infraorbital region of **A**–**E**, respectively

TNF-α, and so on. Among these inflammatory factors, the production of IL-6 and TNF-α stimulates phagocytes to release oxidative products and neutrophil collagenase, which further leads to the degradation of the extracellular matrix. Therefore, IL-6 and TNF-α play important roles in UV-induced inflammation and immune responses [17]. Inhibition of IL-6 and TNF-α production can help ameliorate skin damage caused by photoaging. As shown in Fig. 5F, H, the green fluorescence expression was reduced in both treatment groups indicating that the addition of collagen led to an improvement in the inflammatory state of the skin with better antioxidant effects.

Evaluation of Elastin, MMP3, MMP9, TRP-1, and TYR Gene-Related in Collagen Treatment of Photoaging

Elastin is a crucial component of an organism's ECM, synthesized by fibroblasts and existing in the form of elastic fibers. Loss of ECM and abnormal remodeling of elastin are the main pathological manifestations of photoaging in the skin [18]. Genetic detection using qRT-PCR revealed increased gene expression in the treated group compared with the untreated group (n=3), indicating that collagen significantly promotes elastin remodeling (Fig. 6A).

MMPs are responsible for extracellular matrix degradation, with elevated levels of MMP3 and MMP9 being common in skin adaptation to UV irradiation and dermal aging. This can lead to structural and functional changes in the extracellular matrix, resulting in abnormal morphologic changes such as wrinkles and relaxation. Experimental results (n=3) demonstrated that collagen could inhibit the activity of MMP3 and MMP9 overstimulated by UV light, leading to down-regulation of their expression and accelerated repair of the skin barrier (Fig. 6B, C).

During UV irradiation, melanocytes secrete melanin to protect the skin from damage; however, excessive secretion can lead to pigmentation issues affecting aesthetics [19]. TRP-1 and TYR are key proteins regulating melanogenesis by catalyzing tyrosine into melanin. The experimental results (n=3) showed decreased expression of both TRP-1 and TYR genes in the treatment group, reducing melanin production (Fig. 6D, E).

Quantitative Evaluation of Hyp, SOD, MDA, and α-MSH in Collagen Treatment of Photoaging

Hyp is the primary component of collagen fibers and a unique amino acid in collagen. It serves as one of the parameters for evaluating the degree of skin aging, directly reflecting changes in collagen fibers in the dermis [20]. The experimental results (Fig. 7A) demonstrated that all collagen proteins significantly resisted the reduction of Hyp content caused by UVB, confirming their ability to inhibit collagen degradation and promote the formation of new collagen fibers in the skin (n=3).

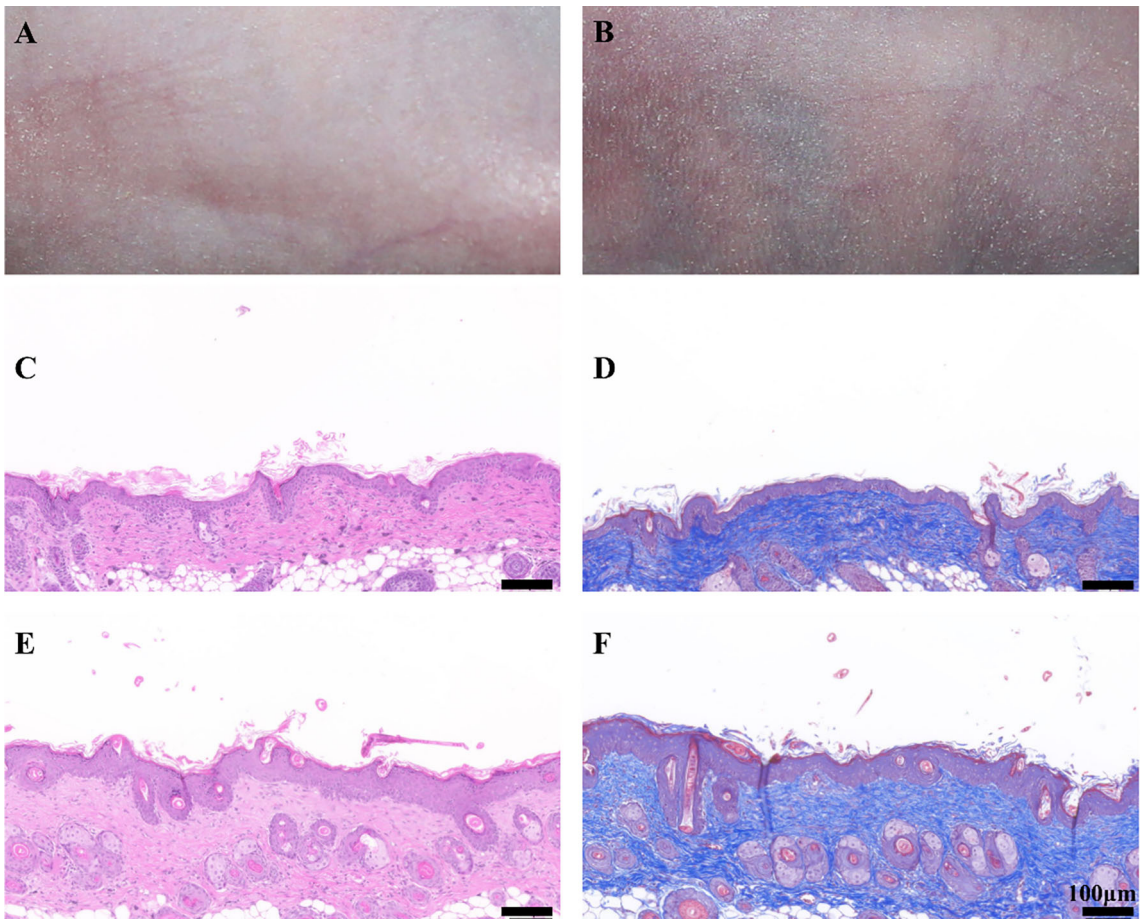

Fig. 3 Preparation of photoaging model. **A** Gross view before model preparation. **B** Gross view after model preparation. **C** HE staining before model preparation. **D** Masson staining before model preparation. **E** HE staining after model preparation. **F** Masson staining after model preparation. Scale bar = 100 μm

SOD is a metal-binding enzyme that catalyzes the conversion of superoxide anion radicals into hydrogen peroxide and oxygen, thereby acting as an antioxidant [21]. The experiment results (Fig. 7B) showed an increase in SOD content after collagen treatment (n=3).

MDA is a product of the reaction between free radicals and lipid peroxidation, which can react with proteins to produce dark-colored substances leading to hyperpigmentation [22]. Its content reflects the degree of lipid peroxidation in organisms and thus indicates cellular damage. Experimental results (Fig. 7C) indicated that collagen inhibited peroxidation processes and ameliorated cellular damage (n=3).

α-MSH, an immunomodulatory peptide consisting of 13 amino acid residues, can act on melanocytes to promote melanin production [23]. The experimental results (Fig. 7D) revealed decreased α-MSH gene expression in the treatment group, resulting in reduced melanin production (n=3).

Discussion

Medical aesthetics is a discipline rooted in the modern medical model, utilizing various methods to assist individuals in appearing younger, more attractive, happier, and more self-assured while also promoting longevity and overall well-being through aesthetic evaluation and comprehensive design. As medical aesthetics has evolved over time, there has been a shift from invasive surgical procedures to minimally invasive techniques that offer reduced complications, lower risks, and clear benefits—making them the preferred choice for those seeking aesthetic enhancements [1]. Currently, injectable treatments such as collagen injections and hyaluronic acid injections dominate the market.

While hyaluronic acid is commonly used, it does carry the risk of vascular embolism which can lead to issues such as skin ulcers or even blindness [24]. Collagen is a biological macromolecule found in animal connective tissue and serves as the most abundant functional protein in mammals with a molecular weight of 300 kD accounting

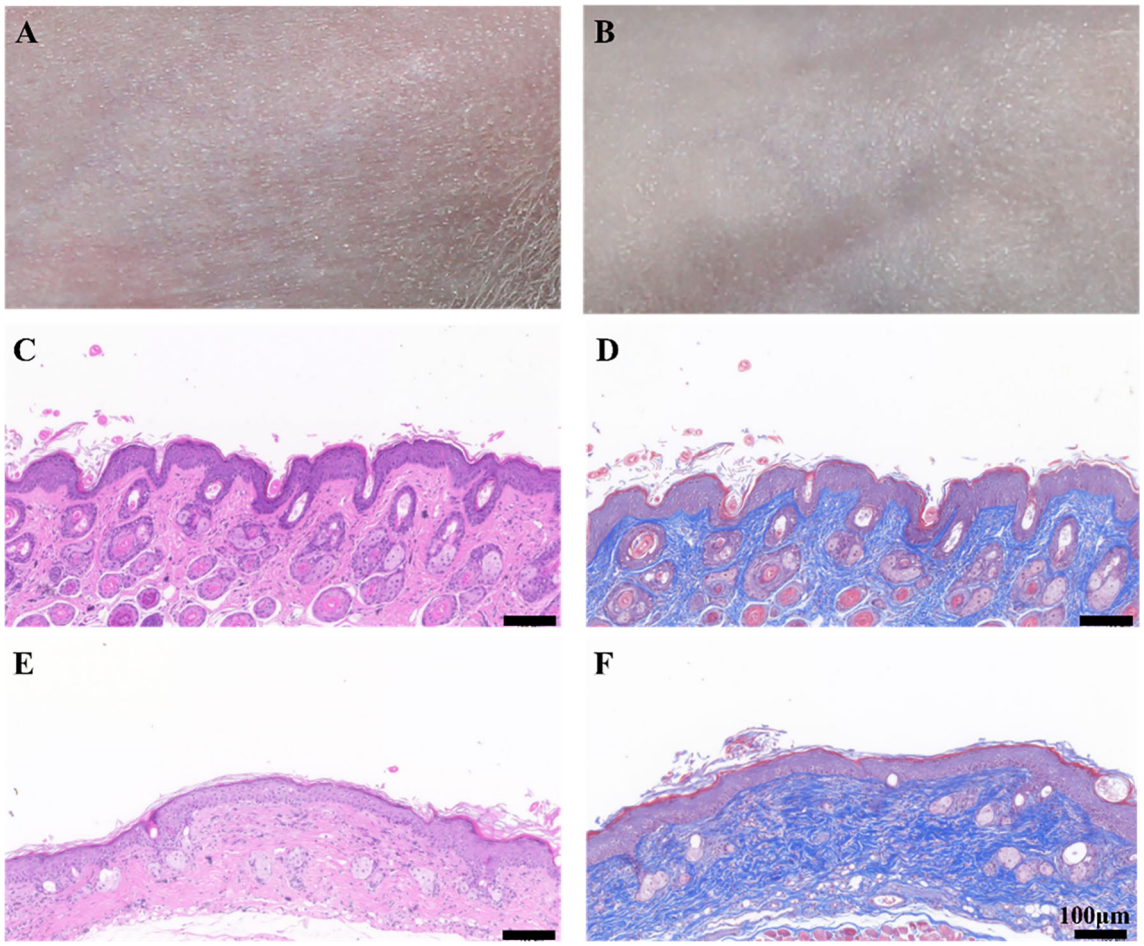

Fig. 4 Assessment of the impact of collagen treatment. **A** Gross view of the untreated group. **B** Gross view of the treated group. **C** HE staining of the untreated group. **D** Masson staining of the untreated group. **E** HE staining of the treated group. **F** Masson staining of the treated group. Scale bar = 100 μm

for 25 to 30% of total protein [25]. The triple helix structure is a common feature of collagen which intertwines into a right-handed helical structure known as a super helical structure. In 1981, Zyderm Type I bovine collagen became the world's first injectable collagen filler approved by the U.S. FDA for improving age-related wrinkles [26].

Among the numerous collagen injectable products, type I and type III composite collagen has been extensively utilized due to its wide range of sources and other advantages. Type I and Type III composite collagen holds great potential in the field of biomedical materials owing to its exceptional biodegradability, biocompatibility, weak immunogenicity, and adaptability to various forms. Additionally, the bionic collagen ratio can promptly replenish the depleted type I and type III collagen in aging skin through volume filling, instantly restoring the disrupted collagen fiber network. Its adhesion and dehydration cohesion can fill subcutaneous tissue defects, thereby achieving therapeutic effects, such as improving skin texture, reducing static lines, enhancing skin laxity, and increasing skin elasticity [25]. Furthermore, the reset reticulation structure provides an anchoring pivot point for cellular tissues which promotes cell proliferation. Moreover, the amino acids produced after material metabolism can serve as raw materials for tissue regeneration. Our findings confirm that type I and type III composite collagen containing type I and type III has a positive impact on improving periorbital aging as well as skin photoaging with a low incidence of adverse reactions; thus, making it a safe and feasible therapy option [25].

Limitations

There are certain limitations to this study. Firstly, this study lacked a longer follow-up of subjects. Despite the rapid degradation time of collagen, its trophic effect on local tissues may persist after degradation. Therefore, a longer follow-up time would allow for further evaluation of its trophic effects. Second, this study lacked quantitative

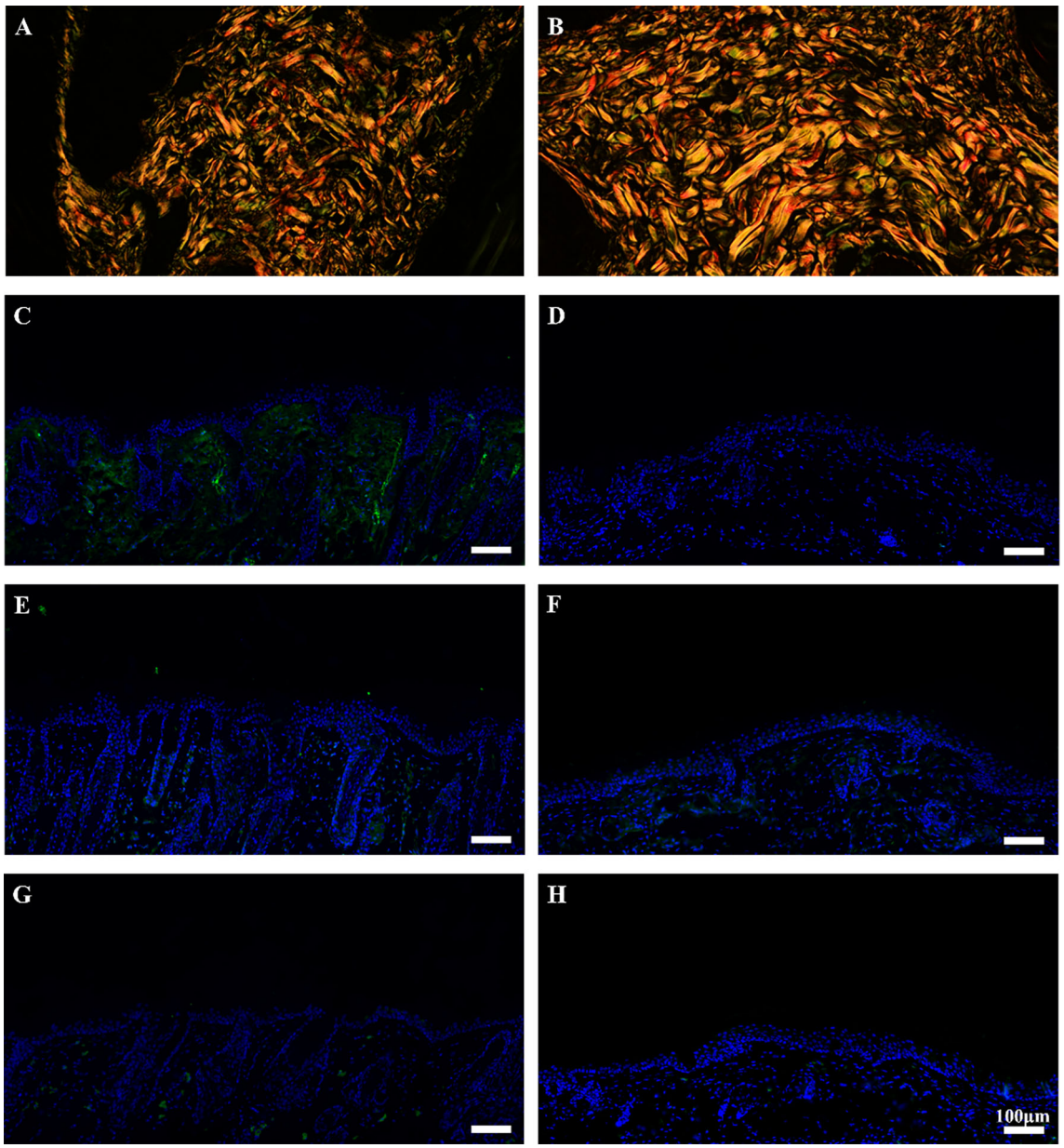

Fig. 5 Evaluation of Sirius red, cellular senescence, and inflammation indexes in collagen treatment of photoaging. **A** Sirius red staining in the untreated group (400x magnification). **B** Sirius red staining in the treated group (400x magnification). **C** Immunofluorescence staining of β-gal cellular senescence in the untreated group. **D** Immunofluorescence staining of β-gal cellular senescence in the treated group. **E** TNF-α in the untreated group immunofluorescence staining. **F** TNF-α immunofluorescence staining in the treated group. **G** IL-6 immunofluorescence staining in the untreated group. **H** IL-6 immunofluorescence staining in the treated group. Scale bar = 100 μm

analysis by VISIA software. As this study only targeted the infraorbital region for injection, the version of VISIA software used in our research center could only perform a full-scale analysis and could not be targeted locally in the infraorbital region, so it was difficult to obtain data specific to the infraorbital region. Third, further in-depth studies should be conducted in nude mice to further elucidate the mechanism of collagen composite in the treatment of photoaging.

Conclusion

Among the various methods of skin anti-aging, type I and type III composite collagen stands out as a promising injectable implant. In comparison to previous inert fillers, type I and type III composite collagen demonstrates an improvement in skin wrinkles and a reduction in the inflammatory state of the skin by replenishing lost collagen and reducing the degradation of collagen and elastin fibers. This study evaluates the impact of collagen on improving

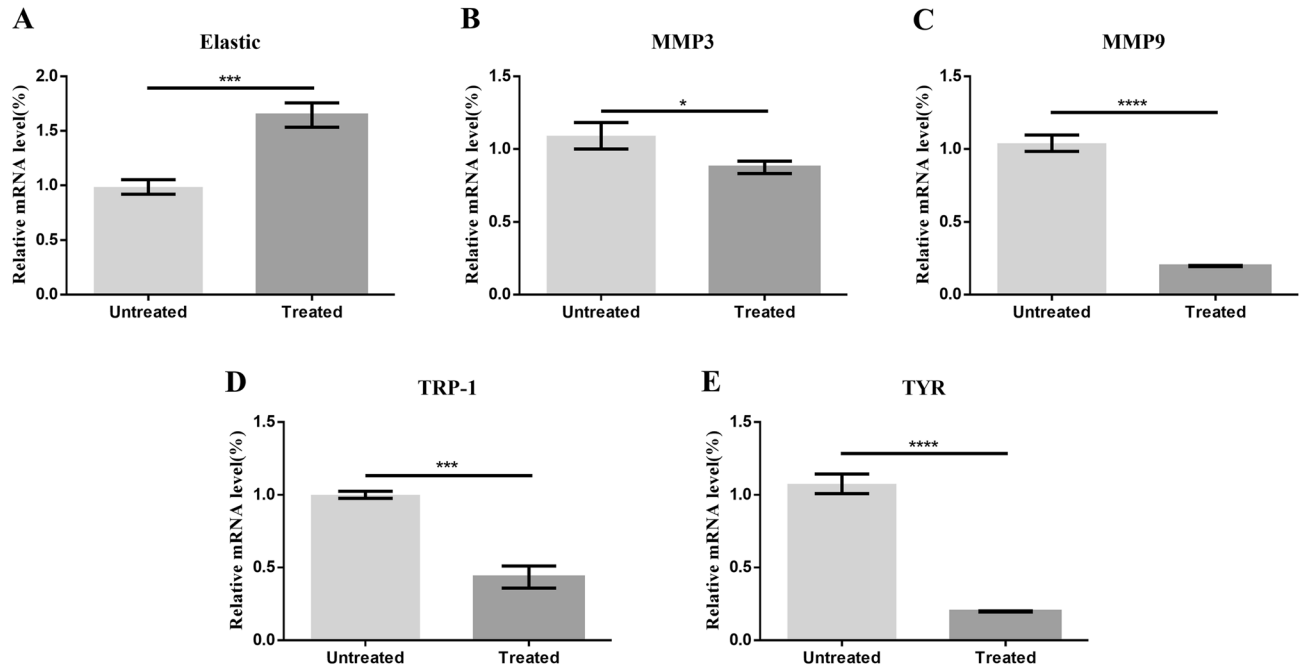

Fig. 6 Gene expression assessment of the effects of collagen treatment for photoaging (**A** $P = 0.0009$, **B** $P = 0.0202$, **C** $P < 0.0001$, **D** $P = 0.0002$, **E** $P < 0.0001$). n=3, $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$

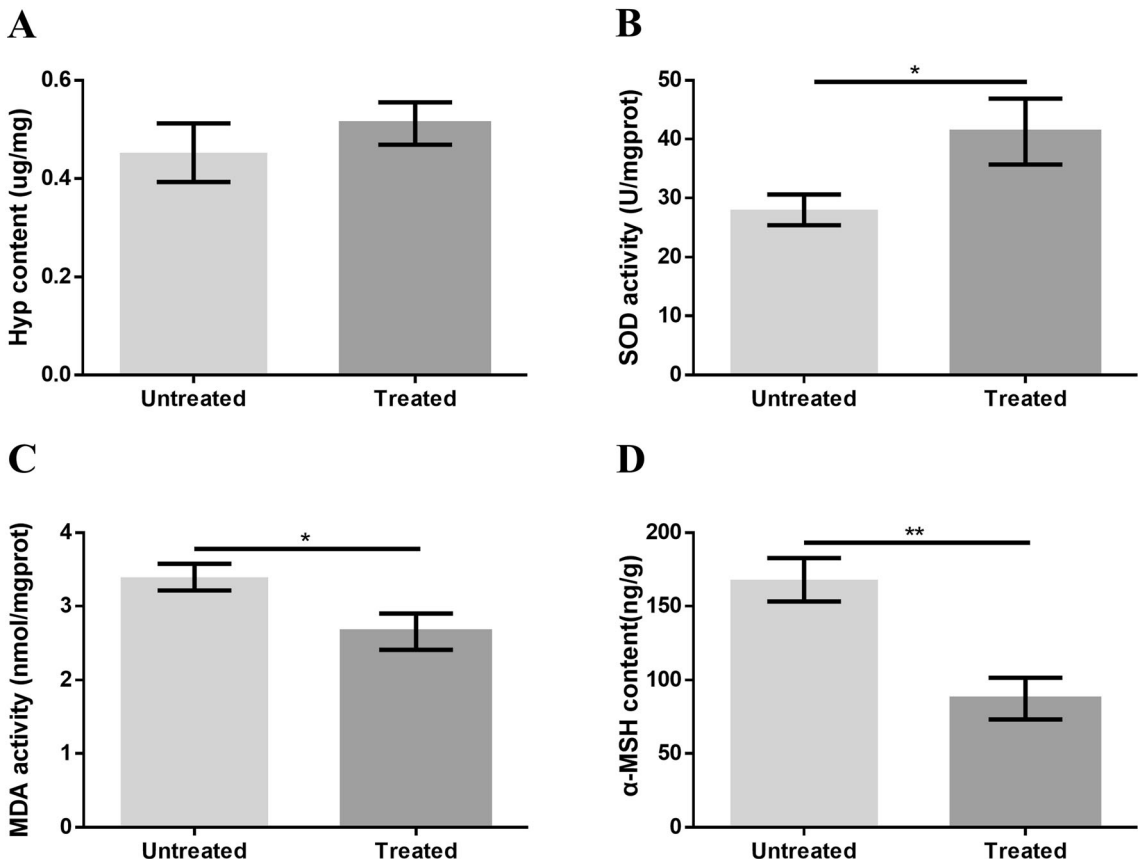

Fig. 7 Quantitative assessment of the effectiveness of collagen in the treatment of photoaging (**A** $P = 0.2335$, **B** $P = 0.0205$, **C** $P = 0.0136$, **D** $P = 0.0024$). n=3, $^{*}P < 0.05$, $^{**}P < 0.01$

infraorbital aging by establishing an animal model of photoaging in nude mice and confirming its biological effect on improving photoaging. The results of this study preliminarily confirm the mechanism of action of type I and type III composite collagen types I and III in improving infraorbital aging as well as treating cutaneous photoaging, affirming its safety and efficacy in collagen remodeling, wrinkle improvement, inflammation reduction, and melanin inhibition.

Author contributions The work described in this manuscript is original and has not been submitted to other journals. All authors have read and approved the submitted manuscript, the manuscript has not been submitted elsewhere nor published elsewhere in whole or in part. All authors have contributed to this study and approved this document and its submission to your journal.

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Declarations

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Ethical Approval All procedures performed in studies involving human participants and animals were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Complete written informed consent was obtained from the patient for the publication of this study.

References

1. Liew S, Wu WTL, Chan HH, et al. Consensus on changing trends, attitudes, and concepts of asian beauty. Aesthetic Plast Surg. 2020;40:193–201. https://doi.org/10.1007/s00266-020-01808-w.
2. Ratajczak P, Maciejak O, Kopciuch D. Directions of hyaluronic acid application in cosmetology. J Cosmet Dermatol. 2023;22:862–71. https://doi.org/10.1111/jocd.15485.
3. Li K, Meng F, Li YR, et al. Application of nonsurgical modalities in improving facial aging. Int J Dent. 2022;8332631:18.
4. Cui H, Zhao H, Xu H, et al. An innovative approach for facial rejuvenation and contouring injections in Asian patients. Aesthetic Surg J Open Forum. 2021;3:1–6. https://doi.org/10.1093/asjof/ojaa053.
5. Franco AC, Aveleira C, Cavadas C. Skin senescence: mechanisms and impact on whole-body aging. TRENDS Mol Med. 2022;28:97–109.
6. Chang L, Fan W, Yuan H, et al. Role of umbilical cord mesenchymal stromal cells in skin rejuvenation. NPJ Regen Med. 2024;9:20. https://doi.org/10.1038/s41536-024-00363-1.
7. Liu Q, Guo L, Zhu Y, et al. Prospective comparative clinical study: Efficacy evaluationof collagen combined with hyaluronic acid injections fortear trough deformity. J Cosmet Dermatol. 2024;23:1515–932.
8. Scotto M, Sagnelli M, Tortora G, et al. The utility of the high-resolution ultrasound technique in the evaluation of autologous adipose tissue lipofilling, used for the correction of postsurgical, post-traumatic and post-burn scars. Radiol Med. 2016;121:521–7. https://doi.org/10.1007/s11547-016-0621-x.
9. Brandt FS, Cazzaniga A, Baumann L, et al. Investigator global evaluations of efficacy of injectable Poly-L-lactic acid versus human collagen in the correction of nasolabial fold wrinkles. Aesthetic Surg J. 2011;31:521–8. https://doi.org/10.1177/1090820X11411161.
10. Cao L, Zhang Z, Yuan D, et al. Tissue engineering applications of recombinant human collagen : a review of recent progress. Front Bioeng Biotechnol. 2024;12:1–16. https://doi.org/10.3389/fbioe.2024.1358246.
11. Wang Q, Yan H, Yao L, et al. A highly bioactive THPC-cross-linked recombinant collagen hydrogel implant for aging skin rejuvenation. Int J Biol Macromol. 2024;266: 131276.
12. Gao J, Guo Z, Zhang Y, et al. Age-related changes in the ratio of Type I/III collagen and fibril diameter in mouse skin. Regen Biomater. 2023;10:rbac110.
13. Wang K, Deng Y, He Y, et al. Protective effect of mycosporine-like amino acids isolated from an antarctic diatom on UVB-induced skin damage. Int J Mol Sci. 2023;24:15055.
14. Salminen A, Kaarniranta K, Kauppinen A. Photoaging: UV radiation—induced inflammation and immunosuppression accelerate the aging process in the skin. Inflamm Res. 2022;71:817–31. https://doi.org/10.1007/s00011-022-01598-8.
15. Xu P, Xin Y, Zhang Z, et al. Extracellular vesicles from adipose-derived stem cells ameliorate ultraviolet B-induced skin photoaging by attenuating reactive oxygen species production and inflammation. Stem Cell Res Ther. 2020;11:264.
16. Xia Y, Zhang H, Wu X, et al. Resveratrol activates autophagy and protects from UVA—induced photoaging in human skin fibroblasts and the skin of male mice by regulating the AMPK pathway. Biogerontology. 2024;25:649–64. https://doi.org/10.1007/s10522-024-10099-6.
17. Shu P, Li M, Zhao N, et al. Efficacy and mechanism of retinyl palmitate against UVB-induced skin photoaging. Front Pharmacol. 2023;14:1278838. https://doi.org/10.3389/fphar.2023.1278838.
18. Watson REB, Gibbs NK, Griffiths CEM, Sherratt MJ. Damage to skin extracellular matrix induced by UV exposure. Antioxid Redox Signal. 2014;21:1063–77. https://doi.org/10.1089/ars.2013.5653.
19. Lee SJ, Kim JE, Choi YJ, et al. Antioxidative role of hygrophila erecta (Brum. F.) Hochr. On UV-induced photoaging of dermal fibroblasts and melanoma cells. Antioxidants. 2022;11(7):1317. https://doi.org/10.3390/antiox11071317.
20. Li P, Wu G. Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. Amino Acids. 2018;50:29–38. https://doi.org/10.1007/s00726-017-2490-6.
21. Li W, Wang S, Li J, Wang X, Cui L, Chen J, Liu Z. Antioxidative enzyme activities in the Rhodeinae sinensis Gunther and Macrobrachium nipponense and multi-endpoint assessment under tonalide exposure. Ecotoxicol Environ Safe. 2020;199:110751.
22. Gil P, Casado A. Malondialdehyde: a possible marker of ageing. Gerontology. 2002;48:209–14.
23. Shi Y, Zeng Z, Liu J, et al. Particulate matter promotes hyperpigmentation via AhR/MAPK signaling activation and by increasing α-MSH paracrine levels in keratinocytes. Environ Pollut. 2021;278: 116850. https://doi.org/10.1016/j.envpol.2021.116850.
24. Zhang L, Zhou Q, Xu H, Gu Q. Long-term prognosis of vision loss caused by facial hyaluronic acid injections and the potential

approaches to address this. Aesthetic Surg J. 2023;43:484–93. https://doi.org/10.1093/asj/sjac329.
25. Liu H, Dong J, Du R, et al. Collagen study advances for photoaging skin. Photodermatol Photoimmunol Photomed. 2024;40:e12931. https://doi.org/10.1111/phpp.12931.
26. Nicolle FV, Ch M. Use of zyderm in the aging face. Aesthetic Plast Surg. 1982;6:193–5.