Improvement of aged skin in Chinese subjects with a cream containing five peptides

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Abstract

Background: Some peptides are developed and utilized in cosmetics, but little in vivo efficacy of the finished cosmetics containing peptides was reported, especially in the Chinese population. Five different peptides as effective ingredients were formulated to evaluate the anti-wrinkle efficacy ex vivo and in vivo.
Methods: Systematic studies were conducted to verify the anti-aging efficacy of the peptides-containing cream. An ex vivo study was performed on human skin explants via topical surface application. In the clinical trial, thirty-one healthy Chinese females with visible facial wrinkles were enrolled and instructed to apply the cream for 8 weeks. Skin aging parameters were measured at 0, 4, and 8 weeks. Subject self-assessments were conducted via questionnaire at each visit.
Results: The results showed the peptides-containing cream treated ex vivo skin produced an increased expression of collagen fibers (collagen I & III) in the dermis, and collagen IV and XVII in the dermal-epidermal junction structure. Global facial anti-aging efficacy was demonstrated by instrumental data and self-assessments. The cheek lines, nasolabial folds, and forehead wrinkles experienced significant reduction to varying degrees at early 4 weeks. At 8 weeks, the dermal density and thickness were significantly increased. The test cream was well accepted by subjects due to its mildness throughout the study.
Conclusion: Collagen content increasing ex vivo and the dermal density and thickness increasing in vivo mutually confirmed its anti-aging mechanism of preventing collagen breakdown and boosting collagen synthesis.
Keywords: peptide; anti-aging efficacy; stimulate collagen production; in vivo; ex vivo

Introduction

Peptides as cosmetic ingredients are comprised of short amino acid chains. They are able to penetrate the upper layer of the skin and act as dispatchers capable of triggering specific functions, such as collagen support so that skin can be firmer, thicker, and more elastic[1-3]. Skin wrinkles are caused by hyperkinesia of skin muscles, and also appear because of the degeneration of extracellular matrix proteins, such as collagen. Depending on the mechanism of action, topical peptides can be classified as signal peptides, carrier peptides, neurotransmitter inhibitor peptides, and enzyme inhibitor peptides[4-7]. As a milder alternative anti-wrinkle ingredient to retinol, peptides have the advantage of high efficacy without the irritant side effects.
Currently, some peptides are developed and utilized in cosmetic products, including natural peptides and synthetic peptides, but little in vivo efficacy of the peptides containing product was reported, especially in the Chinese population[8-10]. A muti-ingredient peptide-based treatment cream has been designed to target the signs of facial aging due to expression lines and photo-damaged skin. Effective ingredients are five different peptides, including acetyl hexapeptide-1, palmitoyl tripeptide-5, hexapeptide-9, acetyl

tetrapeptide-9, and acetyl tetrapeptide-11. Acetyl hexapeptide-1, a new neurotransmitter inhibitor peptide, can identify the optimum amino acid sequence to target presynaptic muscle contraction processes, for a Botox-like activity. Four signal peptides can trigger a signaling cascade and stimulate collagen, elastin, proteoglycan, glycosaminoglycan, and fibronectin production, resulting in skin rejuvenation. Signal peptides have proven effective in reducing wrinkles and improving hydration of tissues, as well as smoothing the skin in in vivo research. Palmitoyl tripeptide-5 mimics the effects of an extracellular matrix protein, thrombospondin-1(TSP-1), a naturally occurring molecule that increases TGF-β activity[5,11,12]. An ex vivo study showed that palmitoyl tripeptide-5 stimulates type I and type III collagen production through the growth factor TGF-β[12]. In a controlled trial, 2.5% Palmitoyl tripeptide-5 demonstrated its anti-wrinkle efficacy and reduced skin roughness better than control groups[4]. Tetrapeptide-9 and tetrapeptide-11 are peptides with the binding of an acetyl group, stimulating the synthesis of type I collagen and basement membrane glycan, urging the growth of human keratinocytes and other advantages[13]. Schagen et al.have found that tetrapeptide-9 andtetrapeptide-11 can make the skin thicker and tighter through clinical research[11]. Hexapeptide-9 stimulates the synthesis of type III and IV collagen expression.

The peptide-based treatment cream with known topical benefits should have a significant increase in collagen expression and improvement in anti-wrinkle results. Systematic studies were conducted to verify the anti-aging efficacy of the peptide-containing cream. An ex vivo study was performed on human skin explants via topical surface application. A clinical trial was conducted on 31 healthy Chinese females with visible facial wrinkles with a period application of 8 weeks.

Materials and Methods

Ex vivo collagen synthesis in human skin tissue

Human skin tissue from a plastic surgery intervention has been utilized in this ex vivo study. The obtained skin tissue was cleaned, cut into small 0.6cm diameter discs, then incubated at 37℃ in a 5% CO2 incubator. The peptide-based cream was treated on the surface of skin tissue for 7 days in the presence of UV irradiation (30 J/cm^2 UVA and 50 mJ/cm^2 UVB) in the early 4 days. The non-treated samples served as a negative control. Before detection, the skin tissue was fixed with 4% paraformaldehyde, embedded, and sliced. The level of collagen fibers of the ex-vivo skin tissue was assessed by Mason staining. The expression of type I, III, IV, and XVII collagen of the ex-vivo skin tissue was assessed by immunostaining. After staining, the slices were photomicrographed and then the level of collagen was analyzed through Image-Pro Plus.

In vivo research

A randomized, double-blind clinical research was carried out from November 2020 to January 2021 in SGS Testing Center Cosmetics. The research protocol (SHCPCH201111050) was examined and approved by the SGS Ethics Committee for Clinical Research. Before enrollment, benefits, risks, and potential complications were explained to the subjects, and informed written consent was obtained from participants. Before clinical research, the cream had passed a 24-h occlusive patch test and proved no adverse effects. Thirty-one healthy Chinese females aged 33-60 (average age 50 ± 5.9 years) with dry skin and conspicuous cheek lines, nasolabial folds, and forehead wrinkles (stratum corneum hydration (SCH) on the check <50 c.u., the clinical score of forehead wrinkles ≥2 according to skin aging atlas, the clinical score of nasolabial folds ≥1 according to skin aging atlas) were screened and enrolled by experienced technicians in the 8 weeks clinical study. Subjects were not pregnant, nursing, or intending to become pregnant during the study. Subjects with skin disease, aesthetical or dermatological treatment

that may interfere with the study, or allergy to cosmetic products, toiletries, and sunscreens were excluded from the study. Subjects were instructed to apply the cream twice daily, in the morning and at night. The usual sunscreen must be cooperatively applied in the daytime. Skin aging parameters were measured at 0, 4, and 8 weeks by the following biophysical techniques and skin image analyses: Corneometer, Cutometer, Ultrascan UC22, Primos-CR, and Visia-CR. Meanwhile, Subject self-assessments were conducted via questionnaire at each visit. All the study procedures were carried out under temperature and humidity-controlled conditions (temperature 21±1℃ and relative humidity 50±10%). Subjects were instructed to clean faces with an assigned cleanser and acclimate to the controlled conditions for 30min prior to measurements.

Skin stratum corneum was measured on the cheek by Corneometer CM 825 (Courage & Khazaka, Germany) .The higher the measured value, the higher the moisture content of the skin stratum corneum. Skin elasticity was evaluated by a noninvasive suction skin Cutometer MPA 580 (Courage + Khazaka, Germany) . The elastic parameter R7 was obtained from skin deformation curves. The closer the R7 parameter is to 1 (100%), skin considers being more elastic. Levels of cheek lines, nasolabial folds, and forehead wrinkles were determined using a 3D roughness analyzer Primos-Lite (LMI Technologies) with the variables of wrinkle number and wrinkle area %. Primos is a non-contact in vivo skin measurement device based on structured light projection. In conjunction with 3D measurement and evaluation software, the system enables to measurement of wrinkle variables. The variable's value decreases with less visible wrinkles, indicating that skin wrinkles were improved. During the measurement, subjects closed their eyes in a relaxed state. Dermal density and thickness were measured by Ultrascan UC22, which is a device used for imaging the skin in high resolution based on 22MHz ultrasound waves entering the skin. The measurement determines parameters such as dermal thickness and dermal density. The value of the parameters increases with more collagen content.

Self-assessments were conducted at each visit at baseline, 4 weeks, and 8 weeks via a satisfaction questionnaire. Subjects were required to assess improvement relative to baseline for hydration, elasticity, firmness, smoothness, cheek lines, nasolabial folds, and forehead wrinkles. Facial skin conditions were assessed using a five-point scale (change from baseline): 1=totally disagree, 2=disagree, 3=neutral, 4=agree, and 5= totally agree. The subjects who agree or totally agree (subjects with a score greater than 3) were counted as satisfaction percentage; the higher the satisfaction percentage, the more people are satisfied. Any cutaneous irritation or adverse reactions would be assessed, recorded, and resolved at each follow-up visit.

Statistical analysis

Data were reported as Mean ± Standard Error of Media(SEM). All statistical analyses were carried out by SPSS. Statistical significance were performed by paired Student's t-test. Results were considered significantly different when $P < 0.05$ (*$P < 0.05$, **$P < 0.01$,***$P < 0.001$). Improvement degree of each parameter was expressed change of percent, which was defined as: Improvement (%) of week 4/8 = [(After treatment value(week4 or week8) - baseline value] / baseline value.

Results

Ex vivo collagen synthesis in human skin tissue

There are 4 types of collagen tested in the study. Collagen I is the most abundant collagen with 80% of the skin's dermis, providing skin strength and integrity. Collagen III is always found together with collagen I, related to skin regeneration and dermis repair. Collagen I &III are the main components of

collagen fibers, accounting for 5-20% of all collagen in the body. Collagen IV forms a network structure to connect the dermis and epidermis and makes the DEJ structure stable; while collagen XVII is transmembrane collagen, which mediates the adhesion of keratinocytes to the underlying DEJ membrane. The density of collagen I&III distribution of the ex-vivo skin tissues was detected by immunohistochemical staining(Fig.1-a & Fig.1-c), while the collagen fiber distribution by Masson staining(Fig.1-e). The density of collagen I&III and collagen fiber distribution significantly decreased after UV irradiation. The treatments with the test peptide-based cream demonstrated a significant increase of the collagen I and collagen III expression in the dermis compared to the UV condition without treatment, by respectively 100% and 22% (Fig.1-b&Fig.1-d). Meanwhile, collagen fiber improved almost by 235% after treatments of the test peptide-based cream in comparison of the UV condition without treatment. According to the results of immunohistochemical observation (Fig.2-a&Fig.2-c), the expression of collagen IV&XVII of the ex-vivo skin tissue decreased significantly after UV irradiation. Compared to the NC group, the treatments with the test peptide-based cream could significantly increase the expression of collagen IV and collagen XVII, by respectively 52% and 132% (Fig.2-b&Fig.2-d). Collectively, test peptide-based cream had a significant repair effect on the decreased collagen density caused by UV irradiation, and on the increased expression of collagen fibers(collagen I &collagen III) in the dermis, and collagen IV and XVII in dermal-epidermal junction structure.

In vivo research

All 31 subjects completed the study and no irritation occurred during the study. Most importantly, the test peptides cream achieved overall anti-aging efficacy within 8-week treatments by instrumental measurement and subject self-assessment. Also,the peptides cream was highly rated on performance and well-accepted on tolerance by subjects throughout the study.

The evaluation results of stratum corneum hydration showed a 29.1% increase after 4 weeks of the test cream application and a 35.5% increase after 8 weeks of application compared with baseline (Fig.3). The stratum corneum hydration data was statistically significant ($p<0.001$). The R7 value representing skin elasticity rarely changed at 2 weeks, and significantly increased by 37.2% ($p<0.001$) at 8 weeks when compared with baseline. (Fig.4). Primos-CR analysis indicated that the cheek lines, nasolabial folds, and forehead wrinkles experienced significant reduction to varying degrees at early 4 weeks in subjects using the test product twice daily. Further improvements in wrinkles were observed at 8 weeks, indicating that the peptide-based cream benefits the entire facial wrinkles. The decrement (p-value) of wrinkle number and wrinkle area (%) at 8 weeks relative to baseline were: for forehead wrinkles 8.8% ($p<0.01$) and 18.7% ($p<0.001$), for cheek lines 17.0% ($p<0.01$) and 23.6% ($p<0.001$), for nasolabial folds 9.2% ($p<0.01$) and 8.1% ($p<0.01$)(Fig.5). The improvement of the skin wrinkles was also confirmed by subject self-assessment. Dermal density and dermal thickness improve synchronously, as the dermal anti-aging markers. Results of dermal density and thickness evaluation both showed a statistically significant gradual improvement during the application of the test cream. Dermal density was increased by 16.7% after 4 weeks' application and 33.2% after 8 weeks' application, compared with baseline, as well as dermal thickness (Fig.6). Collectively, these suggest that the peptide-based cream in this study contributes to the improvement of skin wrinkles and dermal composition. An increase in skin smoothness (90%), firmness (87%), elasticity (81%), and hydration (81%) was reported by subjects. More than 74% of the subjects perceived obvious improvement in the cheek lines, nasolabial folds, and forehead wrinkles (Fig.7).

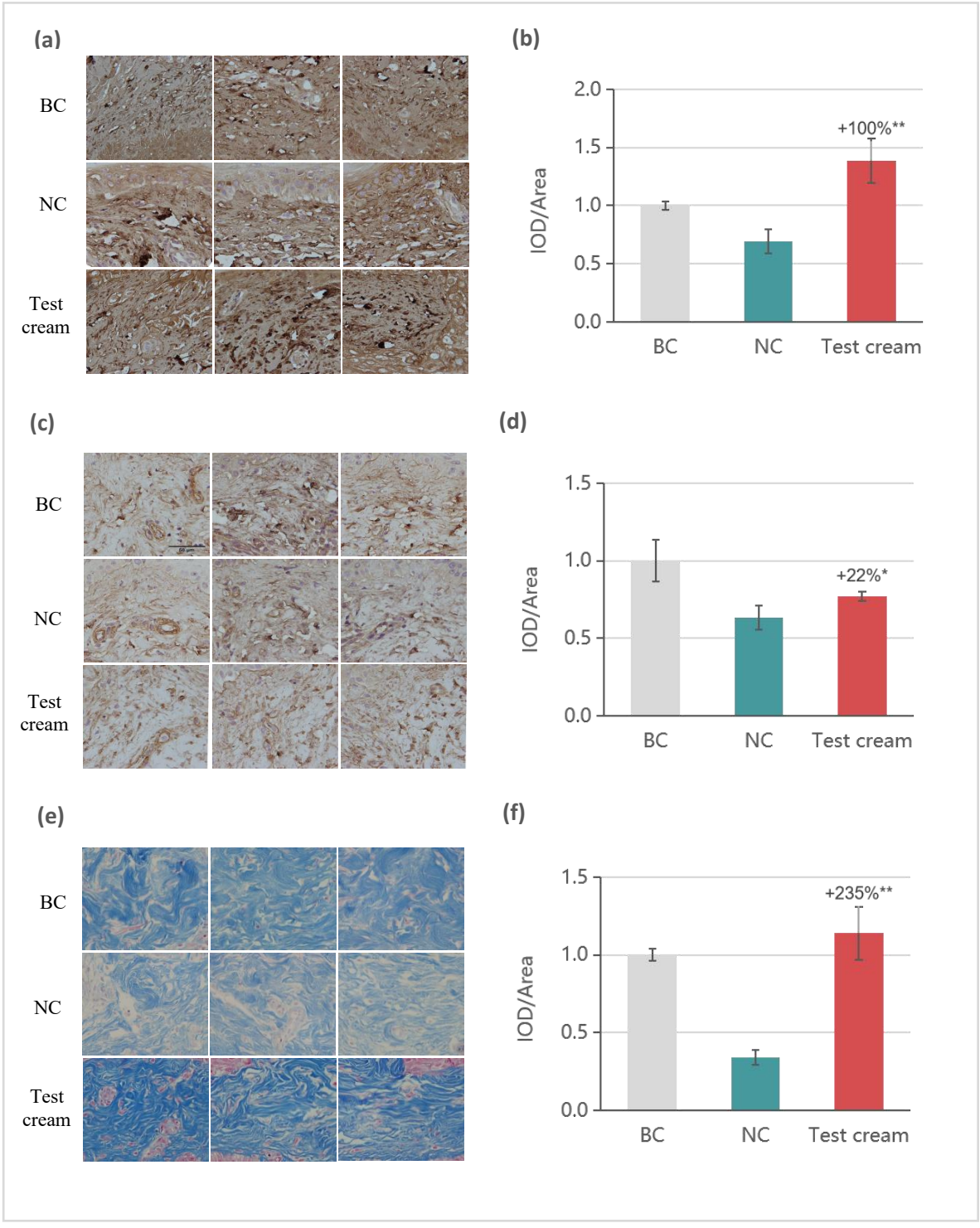

Figure1 Collagen I, collagen III, and collagen fiber expression in the ex-vivo skin tissue. The immunohistochemical staining observation of collagen I(a), collagen III(c), and collagen fiber(e) in the ex-vivo skin tissue irradiated by UV rays. The expression changes of collagen I(b), collagen III(d), and collagen fiber(f) in the ex-vivo skin tissue. Improvement(%) after application of the test cream in comparsion with negative control are presented (**p<0.01 vs. negative control, *p<0.5 vs. negative control).

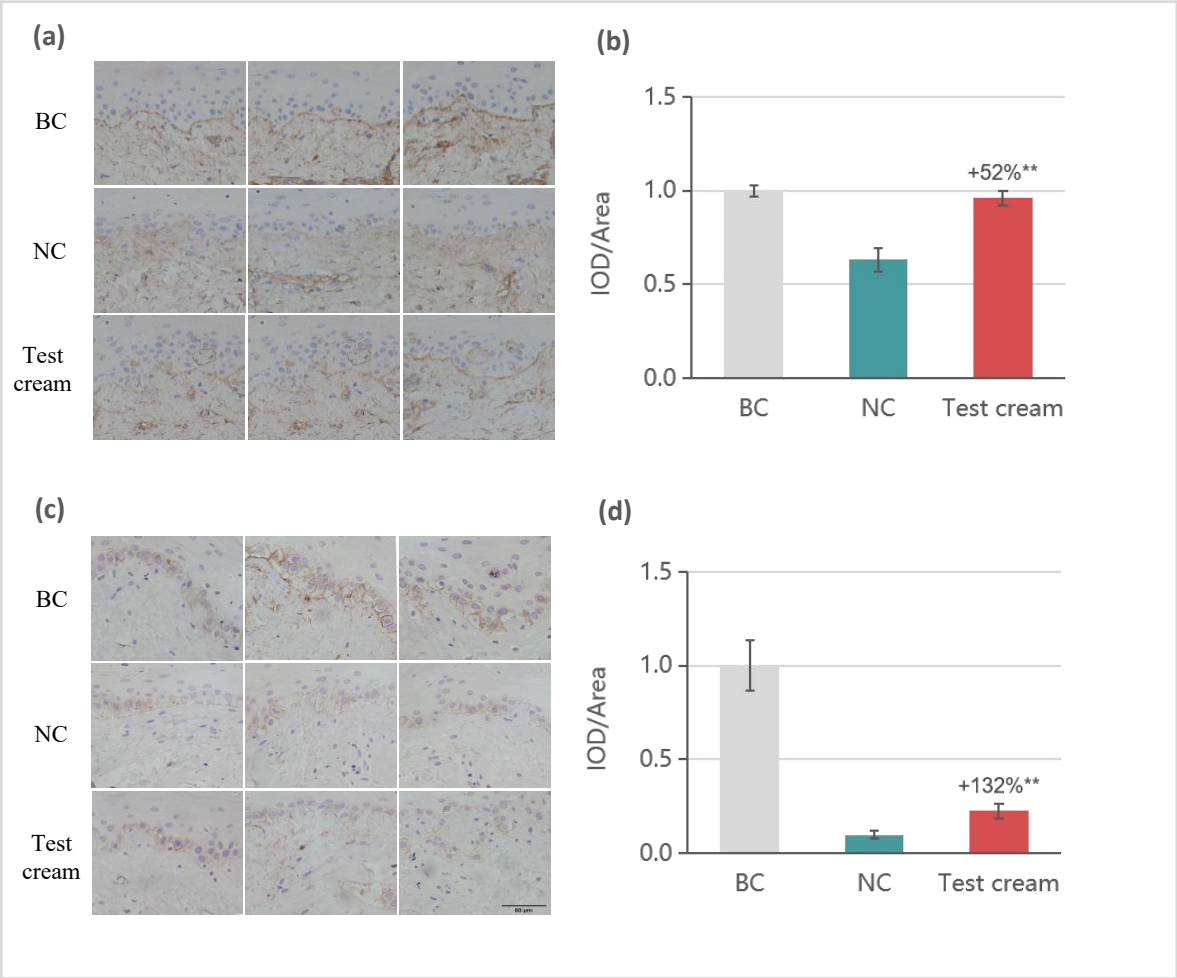

Figure2 Collagen IV and collagen XVII expression in the ex-vivo skin tissue. The immunohistochemical staining observation of collagen IV(a) and collagen XVII(c) in the ex-vivo skin tissue irradiated by UV rays. The expression changes of collagen IV(b) and collagen XVII(d) in the ex-vivo skin tissue. Improvement(%) after application of the test cream in comparsion with negative control are presented (**p<0.01 vs. negative control).

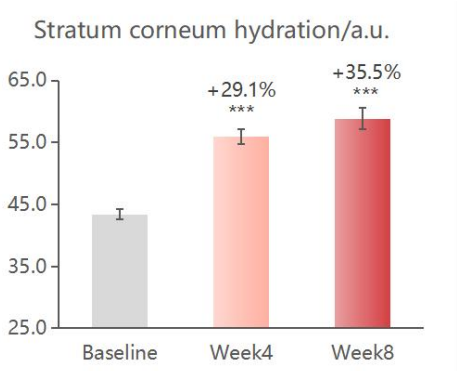

Figure3 Increase of stratum corneum hydration on the cheek after application of the peptide-based cream. All values are expressed as mean ± SEM. Improvement(%) of 4 weeks/8 weeks from baseline are also presented (***p<0.001 vs. baseline).

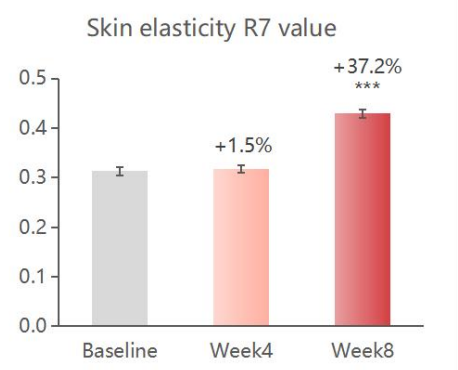

Figure4 Increase of skin elasticity R7 value on the cheek after application of the peptide-based cream. All values are expressed as mean ± SEM. Improvement(%) of 4 weeks/8 weeks from baseline are also presented (***p<0.001 vs. baseline).

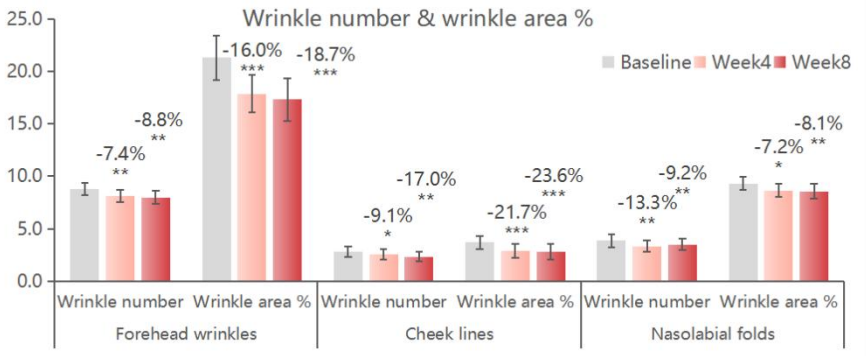

Figure5 Improvement of the cheek lines, nasolabial folds, and forehead wrinkles after application of the peptide-based cream. All values are expressed as mean ± SEM. Improvement(%) of 4 weeks/8 weeks from baseline are also presented (*p<0.05 vs. baseline, **p<0.01 vs. baseline, ***p<0.001 vs. baseline).

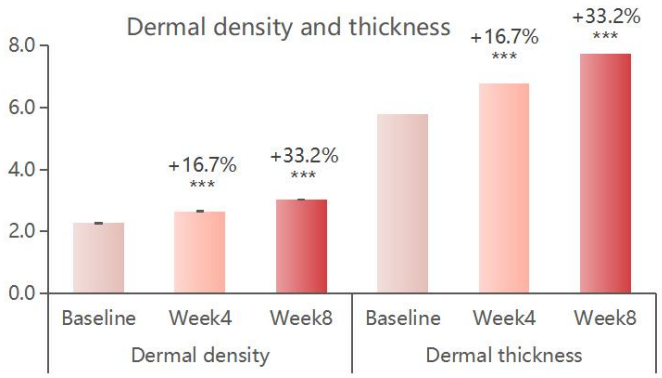

Figure6 Increase of dermal density and thickness after application of the peptide-based cream. All values are expressed as mean ± SEM. Improvement(%) of 4 weeks/8 weeks from baseline are also presented (***p<0.001 vs. baseline).

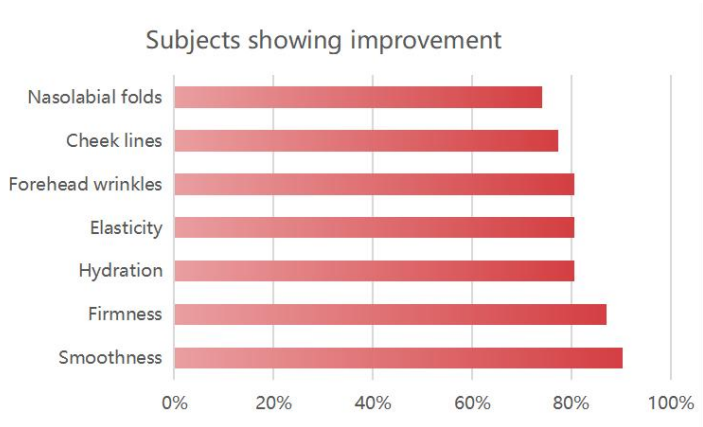

Figure7 Subjects showing improvement (%) in facial skin conditions of smoothness,firmness, elasticity, hydration, cheek lines, nasolabial folds, and forehead wrinkles.

Discussion

Facial skin aging over time, showing signs such as fine lines and wrinkles, increased visual roughness, and reduction in firmness. Collagen breaks down and a decrease in the amount of water held in the epidermis due to both intrinsic and extrinsic factors. Collagens are essential to provide the skin with tensile strength, elasticity, and skin firmness. As people age, the production of various collagens in the skin is reduced. Collagen I and collagen III are the most abundant collagen in the dermis, providing skin strength and integrity. The DEJ (dermal-epidermal junction) structure is flattened and results in collagen fiber loss, leading to fine lines and wrinkles, moisture loss, and sagging skin over time. Our ex vivo study showed a significant increase of collagen I and collagen III expression in the dermis by respectively 100% and 22%, as well as the collagen IV and collagen XVII expression in dermal-epidermal junction structure by respectively 52% and 132%, compared to the NC group.

Global facial anti-aging efficacy was demonstrated by instrumental data and self-assessments throughout the 8-week clinical study from baseline to week 8. The cheek lines, nasolabial folds, and forehead wrinkles experienced significant reduction to varying degrees at early 4 weeks. At 8 weeks, the dermal density and thickness were significantly increased. Collagen content increasing ex vivo and the dermal density and thickness increasing in vivo mutually confirmed its anti-aging mechanism of preventing collagen breakdown and boosting collagen synthesis. The improvement in dermal density and thickness,

therefore, could be attributed to an increase in collagen and elastin fibers because of the main components of the dermis in connective tissues contain collagen and elastic fibers along with cells such as fibroblast, macrophages, and adipocytes. As reported, integrating palmitoyl tripeptide-5 in product formulation provided dermal thickening and density by restoring the natural activity of TSP-1 via triggering TGF-β activation[12]. Palmitoyl tripeptide-5 mimics the sequence located in the protein thrombospondin 1(TSP-1), which has been demonstrated to activate latent tissue growth factor-beta (TGF-β). Other signal peptides incorporated in the formulation were tetrapeptide-9 and tetrapeptide-11. Acetyl tetrapeptide-9 is reported to stimulate collagen type I and lumican synthesis, whereas acetyl tetrapeptide-11 stimulates keratinocyte cell growth and syndecan-1 synthesis. The combination of acetyl hexapeptide-1 in the peptide-based cream binds to the post-synaptic receptors, disrupting the ACh-Receptor cluster and reducing Ca^{2+} release, which is required for muscle contraction. This activity in reducing expression wrinkles has been substantiated through in vivo studies. Hexapeptide-9 stimulates the synthesis of type III and IV collagen expression.

The peptide-based treatment cream with known topical benefits should have a significant increase in collagen expression and improvement in anti-wrinkle results. Systematic studies were conducted to verify the anti-aging efficacy of the peptide-containing cream. An ex vivo study was performed on human skin explants via topical surface application. A clinical trial was conducted 0n 31 healthy Chinese females with visible facial wrinkles with a period application of 8 weeks by instrumental measurement and subject self-assessment. Also the peptides cream was highly rated on performance and well-accepted on tolerance by subjects throughout the study.

Conclusion

This cream consisting of acetyl hexapeptide-1, palmitoyl tripeptide-5, hexapeptide-9, tetrapeptide-9, and tetrapeptide-11 is effective as a mild topical anti-aging formula. In vivo results revealed obvious improvement in the appearance of smoothness, elasticity, cheek lines, nasolabial folds, and forehead wrinkles during this single-center study in 31 women with visible wrinkles. Ex vivo test indicated that the peptide-based cream had a significant repair effect on the decreased collagen density caused by UV irradiation, and on the increased expression of collagen fibers (collagen I &collagen III) in the dermis, and collagen IV and XVII in dermal-epidermal junction structure. Collagen content increasing ex vivo and the dermal density and thickness increasing in vivo mutually confirmed its anti-aging mechanism of preventing collagen breakdown and boosting collagen synthesis. Also, the peptides cream was highly rated on performance and well-accepted on tolerance by subjects throughout the study. For those with mild anti-aging and global rejuvenation needs, this cream provides a good cosmeceutical ingredients solution.

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Conflict of interest

The authors have no other conflict of interest to declare.

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