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Report

Anti-aging effect of an oral disintegrating collagen film: a prospective, single-arm study

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Abstract

Background As the average life expectancy increases, skin aging and wrinkles due to photoaging have gained attention. Collagen is closely involved in the process of skin aging. Among the potential methods of drug delivery to the skin, oral disintegrating films show promise for their ability to bypass the loss of active components that is typical of drug absorption via oral administration. This study was conducted to investigate the effect of an oral disintegrating collagen film on skin aging.

Methods We performed a prospective, single-arm study in a cohort of 22 women to assess the anti-aging effect of a novel oral disintegrating film containing collagen applied daily over a 12-week period. We measured the clinical indicators of skin integrity and performed immunofluorescence and high-performance liquid chromatography analyses of an *ex vivo* oral mucosa model to compare the absorption rates of collagen films and conventional oral tablets via the mucosa.

Results We found that the oral disintegrating collagen film reduced skin wrinkle depth and number and significantly increased skin elasticity and density.

Conclusions The novel mode of delivery of collagen via oral disintegrating films has a clinically potential anti-aging efficacy and is safe and convenient for daily use.

Introduction

As the average life expectancy and income have increased, people have become increasingly interested in aging outcomes, leading to establishment of an aging-related nutraceutical market as one of the largest markets in modern times. Progressive skin aging manifests in various evident phenotypes, including wrinkles, pigmentation, and low skin elasticity.[1] Wrinkles generally form in response to dermal layer collapse, particularly because of collagen deficiency in the skin.[2] Collagen is utilized for its anti-aging effect to increase skin elasticity because it is a major component of the connective tissue, accounting for 30% of the human protein content.[3] Quantitative and structural changes in collagen fibers are among the major modifications observed in the aged skin.[4] Collagen can be consumed by various methods, including direct topical application to the skin surface and oral ingestion in the form of tablets or capsules facilitating absorption through the digestive system and oral mucosa.[5–7] Identifying the most convenient and efficient drug administrative

route is important for maximizing the rejuvenating effect of collagen on the aging skin.

Both topical and oral collagen-containing products have become popular in recent years for their anti-aging properties. Frequently, intact collagen is directly applied on the skin surface, but this has an absorption rate lower than that with other available administrative methods. Absorption through the skin barrier is greatly limited by the product's molecular size, as only small molecules (typically <500 Da) and lipophilic compounds can penetrate the stratum corneum.[8] The limitations of bioactive peptides delivered orally are also well-known; they are unstable in the gastrointestinal tract, poorly penetrate the intestinal mucosa, and are metabolized rapidly in systemic circulation.[9]

Oral drug delivery systems have evolved from conventional oral pills to the most recent oral disintegrating films.[10] Oral disintegrating films utilize a water-dissolving polymer, which allows them to quickly hydrate in saliva and disintegrate within a few seconds, thereby dissolving and releasing the active drug for systemic absorption.[11] Unlike absorption through the digestive system, oral disintegrating films promote direct absorption of the substance into the bloodstream without undergoing systemic metabolism, preventing the reduction of active drug concentration upon reaching the target site. Hence, such films allow application of more accurate amounts of active ingredients than conventional drug formulations and can be easily administered.[12] The ability to control the intake of an accurate amount of active ingredients can reduce the side effects of the high dosages required to bypass drug clearance mechanisms in traditional gastrointestinal delivery. Moreover, traditional oral dosage forms often pose limitations in geriatric, pediatric, and dysphasic patients with various medical conditions.[13]

Oral disintegrating films were first established as a potential drug formulation in 2005, and some pharmaceutical companies in Europe predict that these films will replace approximately 20% of current drug formulations. Indeed, oral disintegrating films are expected to generate more than $200 million in revenue compared with their market value of approximately $5 million in 2007.[14] Therefore, we aimed to investigate the anti-aging effects of a novel oral disintegrating collagen film (Dr. Fill Collagen Film, C.L. Pharm Co., Ltd., Seoul, Korea) and compare these with the effects of other formulations. We found that the application of the oral disintegrating film containing a high concentration of low-molecular weight collagen produced distinct anti-aging effects, as confirmed by the expression of collagen I protein, collagen absorption rate, reduction in wrinkles, and increased skin elasticity and density after 12 weeks of treatment.

Materials and methods

Patients

Twenty-three healthy women aged 20–60 years having noticeable wrinkles around their eyes were enrolled in this study with informed consent. Exclusion criteria included a history of allergies or hypersensitivity, infectious skin diseases, and adverse responses to cosmetics, medicines, or daily light exposure. We also excluded potential participants having lesions on the test site, using medicines with a similar collagen-producing function on the test site, and who underwent any form of dermatologic treatment (e.g., laser, fillers, Botox, and tattoos) in up to 3 months before the study. Among the 23 subjects, one dropped out of the study because of non-compliance with the treatment schedule. Therefore, data were collected and analyzed from 22 participants. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki.

Oral collagen film application and the safety assessment

The oral disintegrating collagen film was composed of 25–35.5% fish collagen (120 mg), pullulan, modified starch, and D-sorbitol/glycerin. The fish collagen or the small-molecular fish collagen peptide was manufactured from tilapia fish scale powder; recent studies suggested that fish scale collagen possesses properties typical of type I collagen consisting of two α1 chains and one α2 chain.[15] Tilapia fish scales are known as one of the best alternative sources for collagen production as they are abundant in type I collagen.[16] The 22 selected participants applied a single collagen film to each side of the lateral buccal mucosa before bed for 12 weeks. To assess the safety and adverse events, all participants were asked to report any adverse events experienced while using the film. The additional analysis on the participant satisfaction after the use of the oral collagen film for wrinkle reduction was performed based on the 5-point global aesthetic improvement scale (GAIS, grade 1 = worse; grade 2 = no change; grade 3 = somewhat improved; grade 4 = moderately improved; grade 5 = very much improved).

Assessment of clinical efficacy

Measurements of visual improvement of periorbital wrinkles

The Antera 3D CS (Miravex, Dublin, Ireland), a device for image acquisition and analysis of skin conditions including wrinkles, was used along with digital photography to measure the depths and numbers of representative periorbital wrinkles. For each participant, periorbital wrinkles (crow's feet) were measured before, and after 4, 8, and 12 weeks of treatment. The "wrinkle-small analysis mode" was utilized to evaluate quantitative changes in the degrees of wrinkles. The number of periorbital wrinkles was measured using Phaseshift Rapid In Vivo Measurement Of Skin (PRIMOS; PRIMOS CR, SnT Lab, Seoul, Korea). The depth (in mm) and count (ea) were expected to decrease as periorbital winkles improved.

Measurements of skin surface elasticity and density

Changes in skin elasticity after applying the collagen film were measured using the Cutometer Dual MPA 580

(Courage + Khazaka Electronic, Köhn, Germany). Moreover, the skin elasticity resilience was determined using a Ballistometer (Dia-Stron, Andover, UK). The coefficient of restitution (CoR) was calculated as the degree of reflection after inflicting the skin with a specific force using a probe tip. Changes in the density of the epidermis and dermis were measured using Ultrascan UC22 (Courage + Khazaka Electronic GmbH) by generating short electrical pulses using a 22-MHz ultrasonic converter. Each measurement was performed thrice during each visit.

Establishment of the *ex vivo* oral mucosa model

The oral mucosa model (SoluOral™) was purchased from Biosolution (Seoul, Korea). This specific model was selected because it has structural and functional similarities with the human oral mucosa and is suitable for assessing collagen absorption rates. In the *ex vivo* study, samples applied to the oral mucosa model were divided into the Control, collagen film treatment (Film), and ground collagen pill treatment (Pill; RAWEL, Seoul, Korea) groups. The collagen pills were composed of 88% low-molecular weight fish collagen peptide (440 mg). Both the oral pills and collagen films were prepared as 2 × 2 mm-sized constructs and cultured with the oral mucosa model in SoluOral growth medium and 20 μl of 1% artificial saliva to compare the absorption rates of collagen based on collagen I expression.

Immunofluorescence staining

To assess the rates of collagen absorption into the oral mucosa, the collagen I expression levels after applying the collagen film or oral collagen pill to the oral mucosa model were measured. The mucosa models were cultured in SoluOral growth medium and incubated with 1% artificial saliva in a humidified atmosphere of 5% CO_2 at 37 °C for 1 hour. After fixation with 4% paraformaldehyde, the mucosa models were processed into 6 μm-thick frozen sections. The frozen sections were then incubated with primary antibodies specific for collagen I followed by incubation with Goat anti-Rabbit IgG H&L secondary antibody (Abcam, Cambridge, UK). Finally, the sections were mounted using VECTASHIELD® mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA) and visualized using a confocal laser scanning microscope (LSM700, Zeiss, Oberkochen, Germany). ImageJ software (NIH, Bethesda, MD, USA) was used to measure fluorescence intensity. All measurements were repeated with three adjacent tissue sections.

Quantification of hydroxyproline using high-performance liquid chromatography (HPLC)

Hydroxyproline was quantified by HPLC to compare the absorption rates of collagen contents between the three groups. The collagen pills and films were prepared as 2 × 2 mm-sized constructs. The oral mucosa model was cultured with the collagen film, collagen pill, or neither in SoluOral growth medium and incubated with 1% artificial saliva in a humidified atmosphere containing 5% CO_2 at 37 °C for 0.5 and 1 hour. After incubation, the hydroxyproline amount in the medium was analyzed by HPLC. The collagen content and absorption rate were calculated as previously described.

Statistical analysis

Data are shown as the mean ± standard error of the mean. All data were statistically analyzed using SPSS Statistics 25.0 software (IBM Corp., Armonk, NY, USA). Data were compared by analysis of variance (ANOVA) followed by repeated measures analysis and *t*-test. Difference was considered as significant when $P < 0.05$.

Results

Patient characteristics

Twenty-three women with wrinkles around their eyes were enrolled in the present study (mean age: 48 ± 3.8 years). However, one participant dropped out due to non-compliance. For the final analysis, data were collected and analyzed from 22 participants.

Clinical efficacy of collagen films

Periorbital wrinkles (crow's feet) were significantly reduced after applying the collagen film (Fig. 1c,e). The wrinkle depth before oral collagen film application was 0.11 ± 0.03 mm, which decreased to 0.09 ± 0.02 mm after 12 weeks of treatment (Friedman test, $P < 0.05$, 20.56% reduction in wrinkle depth, Fig. 1a). Moreover, the number of wrinkles was significantly reduced after using the collagen film (Fig. 1d). In particular, the number of wrinkles significantly decreased after 12 weeks of treatment (27.55 ± 7.63) compared with that before oral collagen film application (37.59 ± 8.95; repeated measures ANOVA, $P < 0.05$, Fig. 1b).

Moreover, 12 weeks of using oral collagen film significantly increased the skin surface elasticity (R2), from 0.66 ± 0.05 before use to 0.75 ± 0.04 after 12 weeks ($P < 0.05$, repeated measures ANOVA, Fig. 2a). The skin elasticity resilience (as indicated by CoR) also improved from 0.67 ± 0.06 to 0.73 ± 0.05 after use ($P < 0.05$, repeated measures ANOVA, Fig. 2b). We also compared the skin densities before and after collagen film use for 4, 8, and 12 weeks (Fig. 3a). The skin density increased after 12 weeks of treatment (65.93 ± 4.35%) compared with that before application (60.42 ± 5.48%; repeated measures ANOVA, $P < 0.05$, Fig. 3b).

Additionally, patients' satisfaction upon the reduction of wrinkles after the use of the oral collagen film was evaluated based on the 5-point GAIS scale. As a result, 17 participants (77.3%) reported as 'moderately improved' or 'very much improved' on periorbital wrinkles after the oral collagen film application. Meanwhile, none reported below 'no change' on the wrinkle

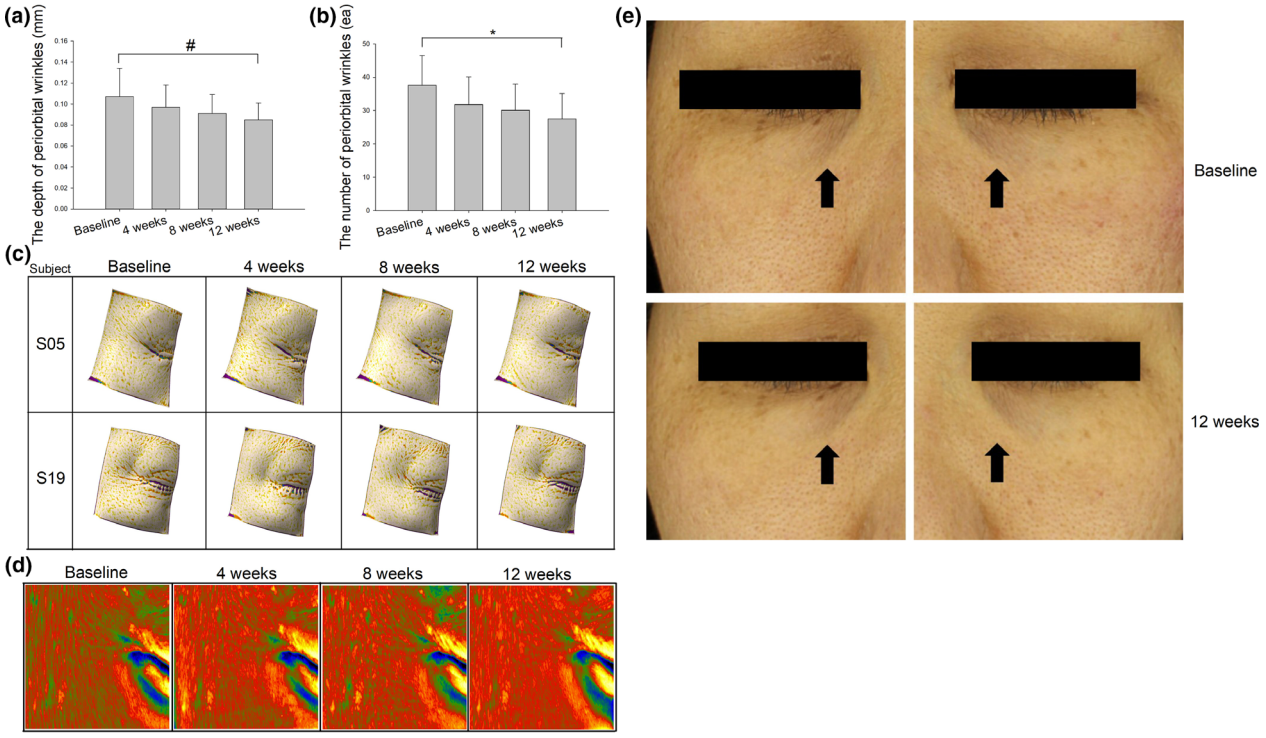

Figure 1 Wrinkle depth and number around the eyes before, and after 4, 8, and 12 weeks of oral collagen film application. Both the wrinkle depth (a) and number (b) decrease in a time-dependent manner in response to the oral disintegrating collagen film (Friedman test, $^{\#}P < 0.05$; repeated measures ANOVA, $^{*}P < 0.05$). The 12-week follow-up images taken with the Antera 3D CS and PRIMOS are shown (c, d). The clinical photographs for comparison of periorbital wrinkle change before and after using oral collagen film (e). The periorbital wrinkles (indicated by black arrows) after using oral collagen film (12 weeks) were a significant improvement compared to before using (Baseline)

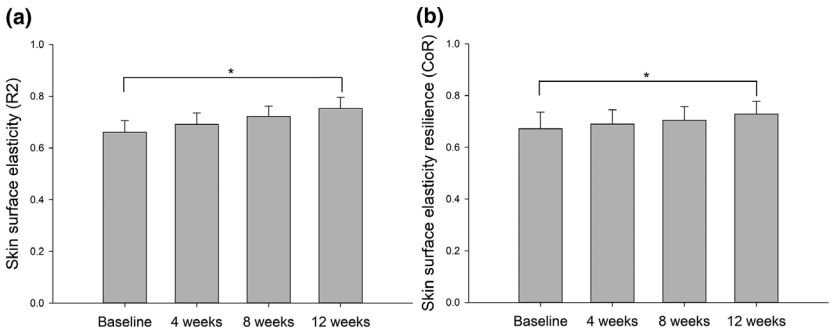

Figure 2 Changes in the skin elasticity (a) and skin elasticity resilience (b) according to the duration of application of the oral disintegrating collagen film. The skin elasticity and skin elasticity resilience improve after the use of the oral disintegrating collagen film compared to those before treatment (repeated measures ANOVA, $^{*}P < 0.05$)

improvement after the end of the study, further emphasizing the clinical efficacy of the study product.

Assessment of the safety and adverse events

There were no reports of adverse events during the 12-week study period; no participants dropped out of the study because of adverse events, suggesting that the oral disintegrating collagen film was safe to use.

Collagen absorption efficacy of oral disintegrating collagen film and oral collagen pills in the oral mucosa model

For *ex vivo* investigation of the increased efficacy of the oral mucosa in absorbing collagen from oral disintegrating films, we applied 2 × 2 mm-sized collagen films or collagen pills to the oral mucosa model. After 1 hour incubation, we performed immunofluorescence staining for collagen I to compare the

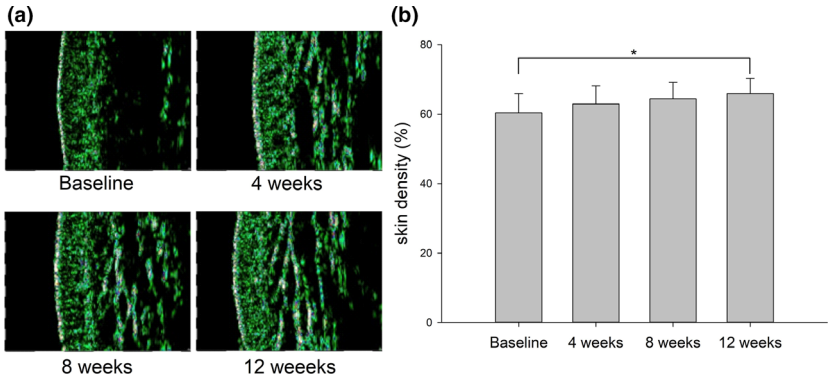

Figure 3 Visualization of the epidermis and dermis densities (a). Percentage of the skin density is calculated by summing the epidermis and dermis densities (b). The skin density increases after 4, 8, and 12 weeks of application of the oral disintegrating collagen film compared with that before treatment (repeated measures ANOVA, $P = 0.017$)

degrees of penetration of collagen components (Fig. 4a). Collagen I expression level was significantly higher, and the product penetrated the mucosa tissue to a greater extent in the Film group than in the Pill group (*t*-test, $P < 0.05$, Fig. 4b).

Evaluation of collagen absorption

Collagen I absorption levels were quantitatively compared among the three groups by HPLC analysis. After 0.5 hours of incubation, the collagen absorption rates of the Pill and Film groups were $0.19 \pm 0.02\%$ and $0.52 \pm 0.02\%$, respectively (independent samples *t*-test, $P < 0.05$, Fig. 5). After 1 hour of incubation, the collagen absorption rates of the Pill and Film groups were $0.26 \pm 0.02\%$ and $1.12 \pm 0.31\%$, respectively (Mann–Whitey *U* test, $P < 0.05$, Fig. 5). Therefore, the oral mucosa absorbed collagen more efficiently from the collagen film than from the collagen pills.

Discussion

Collagen and collagen-derived products are widely used in the pharmaceutical and cosmetic industries for their nutritional and anti-aging properties. Recent studies of collagen from fish processing byproducts have provided potential alternative sources of natural collagen, which is traditionally derived from land-based animals including cows and pigs.[17] When natural collagen is heated, it becomes water-soluble gelatin, a denatured form of collagen, which then undergoes enzymatic hydrolysis to become low-molecular weight collagen peptides.[18] These collagen peptides, abundant in type I collagen, are natural bioactive ingredients showing promise as anti-aging products in cosmeceutical markets. They most often consist of a mixture of peptides of different lengths with a high abundance of the amino acids hydroxyproline, glycine, and proline, which are produced by enzymatic hydrolysis of native collagen.[19] Previous studies demonstrated that the oral consumption of collagen peptides results in increased hyaluronic acid production from dermal fibroblasts and improves the skin barrier function of the stratum corneum.[20,21] Furthermore, collagen peptides can induce collagen synthesis, promoting skin fibroblast proliferation.[22] Some peptides produced from fish skin are shown to scavenge free radicals and reactive oxygen species, therefore serving as potential natural antioxidants.[23,24]

Aging of the human body results in deterioration of multiple structures including the muscles, bones, and skin, and wrinkles are the most prominent sign of aging.[25] The current study investigated the effect of long-term ingestion of oral disintegrating collagen films on the reduction of periorbital wrinkles. The depth and number of wrinkles decreased after using the collagen film for 12 weeks by approximately 20.56 and 26.72%, respectively, indicating that oral disintegrating collagen films can be used to decelerate aging effects. Reduced skin collagen content and destructuring of functional elastic fibers are among the major causes of skin aging; the skin elasticity decreases as aging progresses.[26] We observed that the skin elasticity and resilience increased in parallel with extended use of the oral disintegrating collagen film, further underscoring its anti-aging efficacy. The epidermal and dermal densities of the skin also increased after applying the collagen film, bolstering its anti-aging function.

The novel formulation of collagen supplement as an oral disintegrating film has several advantages over other formulation types including topical collagen application and oral collagen pills. Topical application of collagen-containing skin care products often fails to penetrate the skin barrier and reach the deeper layers of the skin to influence the skin aging process effectively. Choi et al.[27] recently conducted a systemic review of oral collagen supplementation for dermatologic conditions including skin aging; they revealed that oral collagen supplements effectively increase the skin elasticity, hydration, and dermal collagen density without any significant reported adverse events. Since the formulation into films allows for direct collagen absorption without systemic metabolism through the

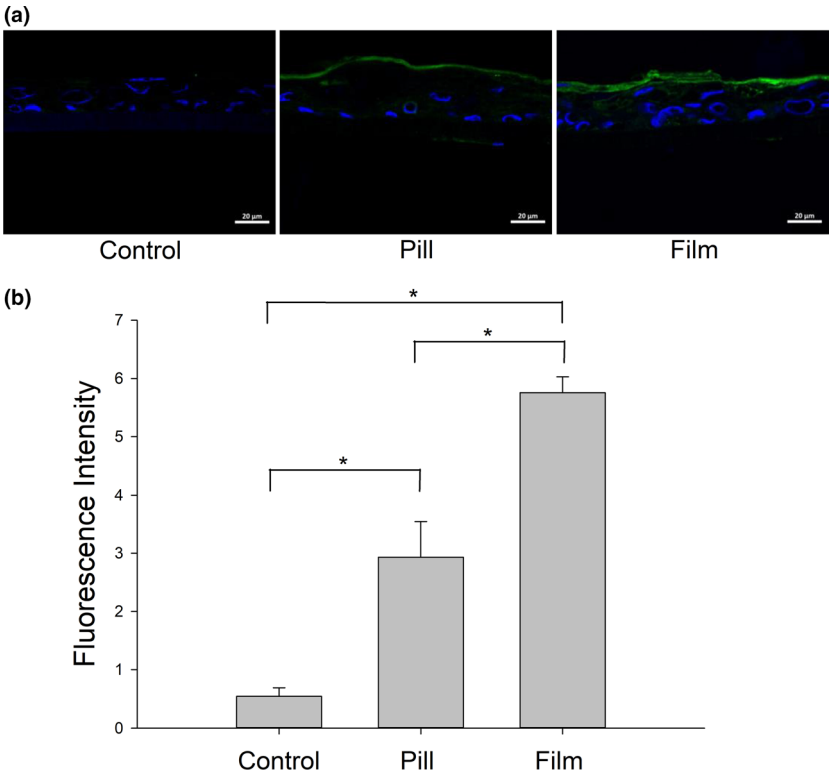

Figure 4 Immunofluorescence staining for collagen I protein in the oral mucosa model (a). Collagen I expression level in the film-treated group (Film) is significantly higher than that in the negative control (Control) and pill-treated (Pill) groups (b, independent samples *t*-test, $*P < 0.05$). Scale bar indicates 20 μm

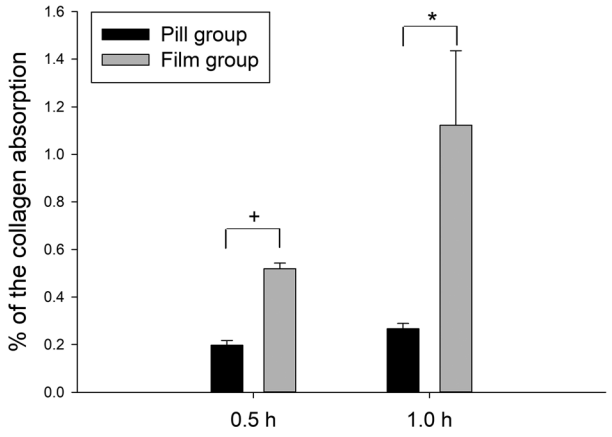

Figure 5 Comparison of collagen absorption between the pill-treated (Pill) and film-treated (Film) groups. Collagen absorption in the Film group increase, in a time-dependent manner, compared with that in the Pill group (independent samples *t*-test, $^{+}P < 0.05$; Mann-Whitey *U* test, $*P < 0.05$)

gastrointestinal tract, it permits the application of accurate and reduced amounts of the active ingredient, thereby reducing drug toxicity and side effects. Although several oral collagen supplementation methods have previously demonstrated their efficacy in improving the skin elasticity and hydration,[28] oral pills inevitably undergo drug loss partially because of the first-pass liver extraction, degradation under a low pH, and protease activities in the gastrointestinal tract.[29] The formulation of collagen into oral disintegrating films is convenient and noninvasive and facilitates the direct entry of the drug into the bloodstream after penetrating the thin oral mucosa. Here, the utilized collagen film led to effective absorption of collagen by the mucosa; the collagen absorption rate was approximately 163.50% higher in the Film group than in the Pill group after 0.5 hours of incubation. After 1 hour, collagen absorption rate in the Film group increased by 321.87% compared with that in the Pill group. Our *ex vivo* data suggested that oral disintegrating collagen films were likely to show improved efficacy over collagen pills pertaining to direct absorption through the oral mucosa into the bloodstream.

As the use of the oral disintegrating collagen film was not associated with any adverse events during the study, our results suggested that this film was likely a safe formulation with significant utility for both cosmetic and pharmaceutical applications. Previous reviews showed that the induction of oxidative stress following collagen synthesis is a major concern for the clinical feasibility of collagen intake.[30] The collagen film, in contrast to oral pills, allows direct absorption of hydrolyzed collagen peptides into the body via the bloodstream without undergoing

gastrointestinal drug metabolism. Hence, less collagen is needed to generate collagen films, and successive adverse effects due to oxidative stress from collagen synthesis can be reduced.

Nonetheless, our study has several limitations. First, it was a single-arm study with a small number of female participants. Further randomized placebo-controlled studies with a larger study population are necessary to advocate the benefits of collagen films. Second, our *ex vivo* data were limited to showing the effective absorption of type I collagen from the collagen film into the oral mucosa model. Additional *in vitro* and *ex vivo* assays showing the expression of senescence-related markers, including senescence-associated inflammatory cytokines and matrix metalloproteinases (IL-8, MMP-3), are recommended to broaden our understanding of the benefits of oral disintegrating collagen films in anti-aging treatments before applying the novel formulation in clinical settings.

Conclusions

Our study revealed that an oral disintegrating collagen film effectively reduced periorbital wrinkles and significantly increased the skin elasticity and density. The *ex vivo* study supported the clinical data, showing greater absorption and higher expression level of collagen I with the collagen film than with the collagen pill. Further randomized placebo-controlled clinical studies focused on the long-term treatment outcomes are required to support the anti-aging efficacy of oral disintegrating collagen films.

Author contributions

S.G.L. and Y.L. conceived and performed the experiments, collected and analyzed the data, and drafted the manuscript. E.B.K., I.J., and J.S. performed the experiments and analyzed the data. Y.L., S.G.L., and J.K. participated in data analysis. J.K and J.H.L. conceived and designed the project, oversaw data collection and interpretation, wrote the manuscript, and shared responsibility for the final content. All authors approved the final manuscript.

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