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Dose-dependent effects of oral yak skin collagen hydrolysate in preventing subacute skin aging

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Abstract

Background: Subacute skin aging is one of the most visible manifestations of overall bodily aging, resulting in skin roughness, oxidative stress and DNA damage. Various treatments for subacute aged skin have been developed; however, they suffer from poor stability, limited efficacy and serious side effects. Yak skin collagen has been reported to effectively repair photoaged skin. **Methods:** In this study, we for the first time investigated the effect of yak skin collagen hydrolysate (MYCH) dose and its oral solution on the prevention of subacute aged skin by constructing a D-galactose-induced subacute aged mouse skin model. The oral solution was formulated using the MYCH, as the efficacious ingredient. **Results:** DermaLab combo evaluation indicated different doses of MYCH and its oral solution contributed to restore the density and skin barrier of subacute aged skin to normal conditions. The repair efficacy of MYCH was positively correlated with its dosage. The repair effect of oral solution outperformed commercially available products. Histological analysis showed that different doses of MYCH and its oral solution could improve the pathological changes in subacute aged skin tissue, restoring the skin structure to normal levels and significantly accelerating collagen fiber regeneration. Furthermore, antioxidant index analysis in skin tissue indicated that different doses of MYCH and its oral solution help reduce oxidative stress, with the oral solution showing significant advantages. Cell-based experiments showed that MYCH and its oral solution could inhibit the expression of ECM-degrading enzymes MMPs, providing molecular-level support for the maintenance of matrix homeostasis. **Conclusion:** These findings suggest that the oral product made with MYCH can effectively repair and improve skin condition, offering broad application prospects in the fields of nutritional supplements and skincare products.

Keywords: yak skin; collagen hydrolysates; Oral solution; subacute aged skin

Author contributions

Zhang-Wen Liu: Validation, Data Curation, Project administration, Visualization, Methodology. Na Li: Data Curation, Validation, Investigation, Methodology, Visualization. Wen-Hua Li: Project administration, Methodology. Lin-Yan Yao: Supervision, Writing - Original Draft, Writing - Review & Editing, Funding acquisition. Jian-Xi Xiao: Conceptualization, Funding acquisition, Supervision, Project administration, Writing - review & editingAll authors discussed the data and contributed to editing the manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

MYCH: Medium Yak Collagen Hydrolysate; MMP-2: Matrix Metalloproteinase -2; MMP-12: Matrix Metalloproteinase-12; SOD: Superoxide Dismutase; MDA: Malondialdehyde; MWDECGS:Molecular Weight-Directed; Enzymolysis-Chromotography Green Synthesis; ECM: Extracellular Matrix; UV: Ultraviolet.

Citation

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Introduction

Skin aging is one of the most visible manifestations of the body's aging and is an inevitable physiological process [1–3]. Based on the rate of aging, skin aging can be categorized into two types: acute aging and subacute aging. Subacute skin aging is a common type of skin aging, usually caused by prolonged exposure to ultraviolet (UV) radiation [4–5], continuous excessive sugar intake [6], and long-term exposure to heavily polluted environments [7–9]. Among these factors, the long-term intake of sugars such as D-galactose is considered as one of the main causes of subacute skin aging. The main manifestations of subacute skin aging include rough skin, fine lines, sagging, and yellowing. The aging mechanism induced by D-galactose mainly involves the reactive oxygen species (ROS) and advanced glycation end products (AGEs) produced during its metabolism in the body. These substances can lead to oxidative stress and DNA damage, activating inflammatory responses and apoptosis pathways, thereby accelerating the skin aging process [10–12].

Various treatments for subacute skin aging have been developed including topical application of vitamin A [13–14], radiofrequency [15–16], and dermal fillers [17–18]. Weinstein et al. found that continuous use of 0.05% retinoids for six months improved the skin condition of subjects, including reducing roughness, sagging, and hyperpigmentation [19]. However, vitamin A and its derivatives are quite irritating to the skin [20]. Radiofrequency works by directly delivering uniform heat to the dermis, which immediately promotes collagen contraction and regeneration, thereby improving skin wrinkles and laxity [21–22]. This method is associated with noticeable pain during treatment [23–24] and requires specialized equipment. Dermal fillers restore the appearance of the skin by filling in wrinkles and sagging areas, causing adverse reactions such as erythema and pain [25].

Oral administration of hydrolyzed collagen has been found to promote collagen synthesis in the skin, increase the quantity of collagen fibers, thereby aiding in the prevention and treatment of skin aging. Liang et al. found that long-term oral of hydrolyzed collagen from salmon skin significantly increased the expression levels of type I and type III collagen in the skin of rats [26]. Ohara et al. demonstrated that daily oral intake of fish collagen hydrolysate for four weeks effectively increased the moisture content of the stratum corneum in the subjects [27]. Czajka et al. found that long-term oral of a liquid supplement containing hydrolyzed fish collagen, vitamins, and other active ingredients contributes to improve the skin condition, mainly by enhancing skin elasticity and hydration [28].

Yaks, the species native to high-altitude regions, provide collagen with excellent biocompatibility and biological activity. Previous studies have demonstrated that yak skin collagen contributed to the repair of photoaged skin. In this study, we for the first time investigated the reparative effects of different doses of medium molecular weight yak skin collagen hydrolysate (MYCH, 1000 Da < M < 3000 Da) on aging skin and further developed an oral product containing hydrolyzed collagen from yak hide (Figure 1). By establishing a D-galactose-induced subacute aging mouse skin model, the reparative effects of different doses of MYCH and its oral liquid product on aging skin were investigated. DermaLab combo evaluation, histological analysis, and antioxidant index results indicated that with the increase in MYCH dose, its reparative effects gradually enhance. In addition, the oral product formulated with MYCH demonstrated significant advantages over commercially available hydrolyzed collagen oral products in reducing skin wrinkles, increasing skin hydration, enhancing skin density, and attenuating skin oxidative stress. Therefore, the oral products formulated with MYCH hold promising market prospects in the fields of nutritional supplements and skincare products.

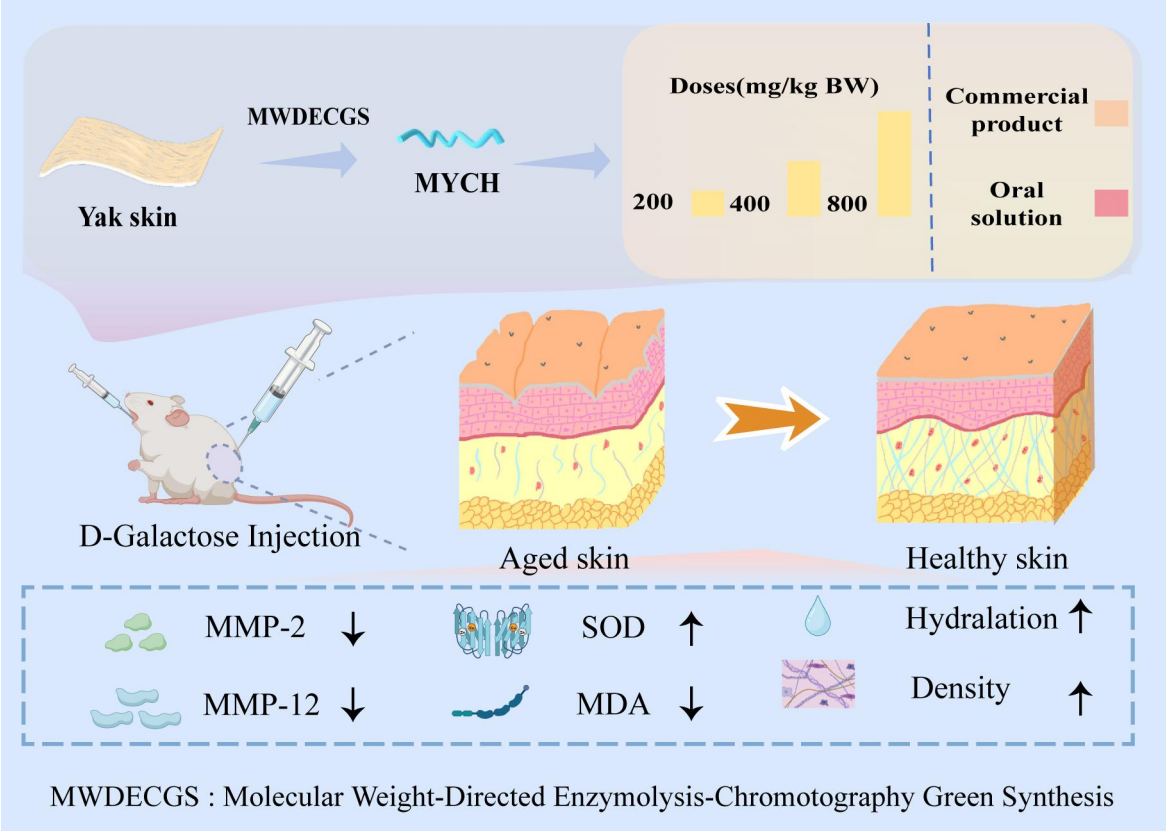

Figure 1 Schematic illustration of oral yak skin collagen hydrolysate with different doses for healing of D-galactose-induced subacute aged skin. MYCH: Medium Yak Collagen Hydrolysate; MMP-2: Matrix Metalloproteinase-2; MMP-12: Matrix Metalloproteinase-12; SOD: Superoxide Dismutase; MDA: Malondialdehyde.

Material and methods

Materials

D-galactose was purchased from Sinopharm Chemical Reagent Co., Ltd. L-hydroxyproline was purchased from Shanghai Yuan Ye Biotechnology Co., Ltd. Commercial kits used for determining superoxide dismutase (SOD) and malondialdehyde (MDA) were purchased from Beijing Solarbio Science & Technology Co., Ltd. All other chemicals used in the study were of analytical grade or better.

Preparation of yak skin collagen hydrolysate

The yak skin (Gansu, China) was thawed at room temperature, and then the hair and subcutaneous tissues were removed from the yak skin and cut into small pieces. Defatting was performed using 10% n-butanol for 12 h, followed by treatment with 1 M NaOH for 11 h to remove non-collagenous components from the yak skin. The yak skin was washed with water until the pH reaches 7.0. Pre-treated yak skin was added to a 0.5 M acitric acid system, then pepsin was added and the reaction was carried out at 25 ˚C for 36 h. After completion of the reaction, filtration was performed, and the pH of the filtrate was adjusted to neutral. Yak skin collagen hydrolysate (MYCH, 1,000 Da < Mw < 3,000 Da) was obtained by adding 80 U/mL of papain to the filtrate and incubated at 50 ˚C for 5 hrs. The enzymatic products were further purified using size exclusion chromatography with a dextran G25 gel column. Dextran G25 gel was pre-treated and packed into a prepared polytetrafluoroethylene glass chromatography column, ensuring column equilibration and stability. 100 mL of the enzymatic products were loaded onto the column, using distilled water as the eluent, and the elution flow rate was controlled at 1 mL/min. During elution, every 5 mL of eluent was collected into separate tubes, and the absorbance at 220 nm was measured to plot an elution curve of absorbance against tube numbers. The molecular weight distribution of the eluent in each tube was determined using size exclusion chromatography, and the target products were collected, freeze-dried, and stored at −20 ˚C. The molecular weight distribution of MYCH has been characterized in our previously study using high-performance liquid chromatography (HPLC). The results demonstrated that peptides with molecular weights ranging from 1,000 to 3,000 Da accounted for more than 50% of the total peptide population. In addition, the degree of hydrolysis (DH) of MYCH was determined to be approximately 40%, indicating a moderate level of enzymatic hydrolysis [29].

Preparation of MYCH oral solution

With MYCH as the main active ingredient (6%), the formulation of the oral solution was designed with reference to the composition and proportion of related commercially available oral products, combined with vitamin C (0.6%), hyaluronic acid (0.04%), bilberry extract (0.2%), supplemented by unique highland plant ingredients (0.03%) including black wolfberry powder, salicin, and other food additives, to develop MYCH oral solution.

Construction of subacute aged skin model

All animal experiments were performed with protocols approved by the ethics committee of School of Life Sciences, Lanzhou University (No. EAF2022010). Three-month-old female Kunming mice weighing 20 ± 2 g were purchased from Lanzhou Veterinary Research Institute (Lanzhou, China). The mice were randomly divided into 7 groups: normal group (Blank), model group (Model), low-dose yak skin collagen hydrolysate group (MYCH-200), medium-dose yak skin collagen hydrolysate group (MYCH-400), the high-dose yak skin collagen hydrolysate group (MYCH-800), commercial product group (Commercial product), and MYCH oral solution group (Oral solution), with 24 mice in each group.

The mice in the blank group received no treatment, whereas those in the other groups were orally administered a daily D-galactose solution at a dose of 500 mg/kg body weight for a duration of two months. Compared to the blank group, the mice administered D-galactose showed a significant decrease in moisture content and skin density on their dorsal skin, along with a significant increase in transepidermal water loss (TEWL), indicating the successful establishment of the D-galactose-induced subacute skin aging model. Then, the mice in the blank and model groups were orally administered 0.4 mL of physiological saline daily. The mice in the MYCH-200, MYCH-400, and MYCH-800 groups were daily gavaged with 0.4 mL MYCH solution at doses of 200 mg/kg body weight (BW), 400 mg/kg BW, and 800 mg/kg BW, respectively. The Commercial product and Oral solution groups mice were each orally administered 0.4 mL of their respective products. Six mice from each group were taken at the 2, 4, 6, and 8 weeks for measurement of various DermaLab Combo indicators. After the measurements were completed, the mice were euthanized, and their dorsal skin tissues were collected for further analysis.

DermaLab combo evaluation

DermaLab combo was applied to non-invasively evaluate the skin condition. DermaLab combo is commonly used skin detection device that combines skin ultrasound, dermatoscopy, and skin physiological indicator detection, which can efficiently detect skin conditions. Skin videoscope, skin ultrasound image, dermis density, hydration, and TEWL, were measured at weeks 2, 4, 6, and 8. Three measure areas evenly distributed on dorsal skin were selected for each mouse.

Histological analysis

Dorsal skin tissues in each group were harvested at weeks 2, 4, 6, and 8. The skin tissue samples were fixed in 4% paraformaldehyde tissue fixative for 72 h and then subjected to paraffin embedding. The tissue sections were 4 μm thick. The tissue sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome staining, respectively, and analysed using an upright fluorescence microscope.

Quantitative analysis of hydroxyproline

Chloramine-T colorimetric method was employed to determinate the content of Hyp in hydrolysates. Briefly, the dorsal skin of the mice was dried in an 85 ˚C oven and cut into fragments. 20 mg of skin tissues were treated with 2 mL of HCl (6 mol/L) at 85 ˚C for 12 h to allow complete hydrolysis of collagen. The pH of the solution was then adjusted to 7.0 at room temperature, and the hydrolysates were diluted to constant volume for further quantitative analysis. Chloramine T was added to the solution and incubated at room temperature for 20 min. The chromogenic reagent was then added and incubated at 85 ˚C for 10 min. The mixture was cooled to room temperature. The UV absorption at 561 nm was measured, which can directly reflect the Hyp content in hydrolysates according to a standard curve of Hyp solution.

Antioxidant indicator analysis

Using colorimetric methods, the activity of superoxide Dismutase (SOD) and the content of malondialdehyde (MDA) were determined. Initially, skin tissue was weighed and added to the extraction solution at a ratio of 0.1 g/mL, followed by homogenization on ice. Subsequently, the homogenate was centrifuged at 8,000 g for 10 mins at 4˚C. The supernatant was collected, and its absorbance was measured using a UV-1750 spectrophotometer, respectively.

RT-qPCR

RT-qPCR analysis was performed to assess the effect of MYCH on cell differentiation. L929 cells (1×10^5 cells/mL) were seeded into well plates and treated with MYCH or the oral solution for 4 days. Total RNA was extracted using an RNA isolation kit. RNA concentration and purity were assessed with a NanoDrop spectrophotometer. cDNA was synthesized using the PrimeScript RT kit with gDNA Eraser (Takara-RR047Q, Japan). Gene expression was analyzed using TB Green Premix Ex Taq II (Takara-RR820Q, Japan) on a real-time PCR system (Agilent, Mx3005P, USA). Relative gene expression levels were calculated using the 2^−ΔΔCT method and normalized to the internal control. Primer sequences for MMP genes are listed in Table 1.

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Table 1 Primer sequences used for RT-qPCR.

Gene	Forward (5'-3')	Reverse (5'-3')
MMP-2	GACCGCTTGGCTTCAAATCA	CCGCATGGTCTCGATGGTAT
MMP-12	ACACCTGACATGAACCGTGAG	TGGCCAAGACCTAAGGAATG

Statistical analysis

The data are presented as the mean ± standard deviation (sd) of three or more experiments and were statistically analysed by student's t-test. For each experimental group, 24 mice were included and evaluated at four time points; at each time point, six mice were randomly selected for non-invasive skin assessments. For ex vivo skin tissue analyses, the sample size was n = 3 per group. Statistical significance was defined as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Results and discussion

Combo non-invasive evaluation the parameters changes of subacute aged skin

The skin videoscope probe of combo was employed to observe the appearance of mice dorsal skin at 2, 4, 6, and 8 weeks (Figure 2a). Throughout the experiment, the skin in the blank group remained smooth; while the dorsal skin in the model group became dry, rough, and noticeably wrinkled. At week 2, compared to the model group, the dorsal skin wrinkles in each experimental group were slightly reduced. By the 4th and 6th weeks, the wrinkles on the dorsal skin of each experimental group were further reduced. Notably, the skin condition of the oral solution group was similar to that of the blank group. By the 8th week, the MYCH-200 group had slightly wrinkled skin, the MYCH-400 and commercial product groups had slightly fine lines. The MYCH-800 and oral solution groups was comparable to the blank group. These results indicate that different doses of yak skin collagen hydrolysate (MYCH, 1,000 Da < Mw < 3,000 Da) and its oral solution can improve the appearance of subacute aged skin. The repair effect improved with increasing doses of MYCH. Additionally, the oral product prepared with MYCH outperformed the commercial product.

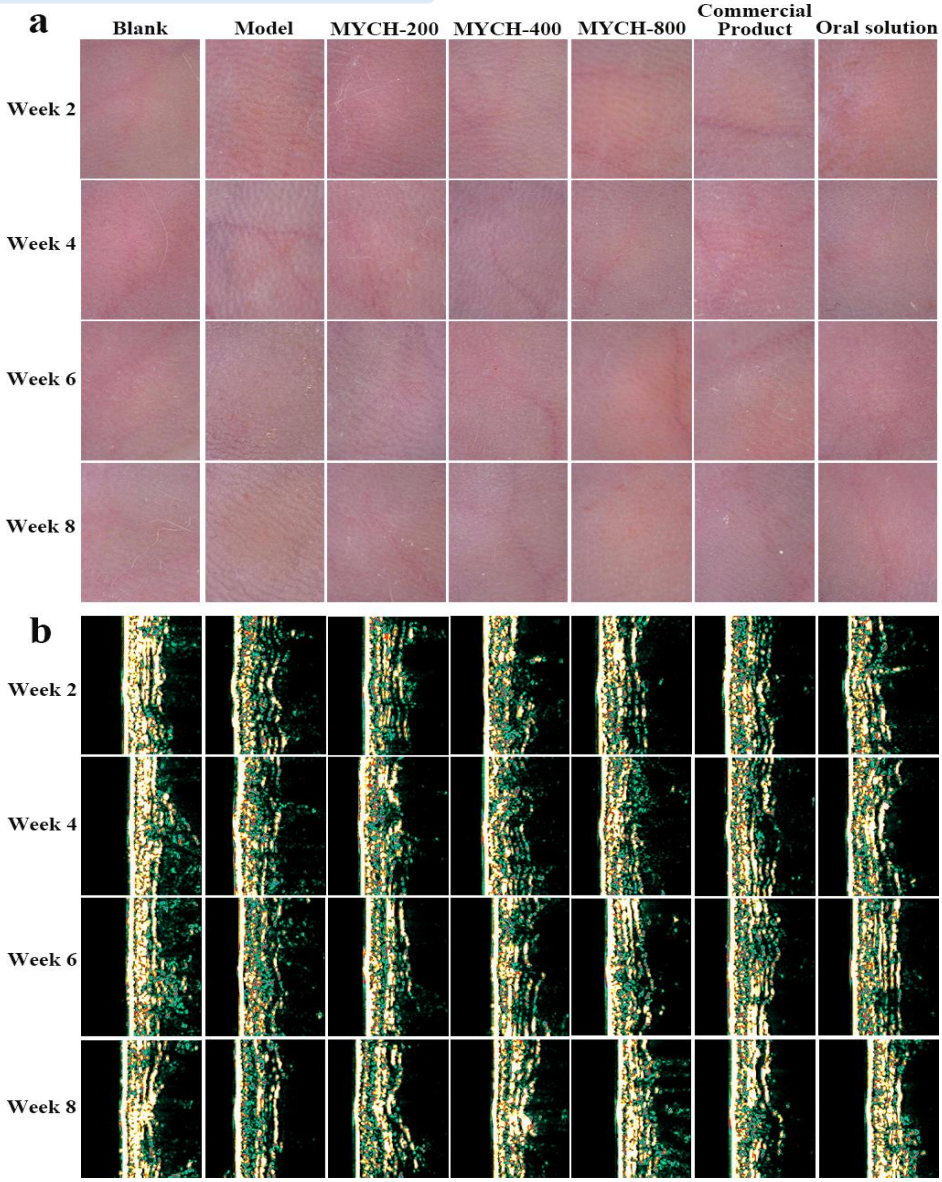

Figure 2 Combo qualitatively evaluated the repair performance of different doses of yak skin collagen hydrolysate (MYCH) and its oral solution for subacute aged skin. Optical images (a) and high-frequency ultrasound images (b) of the mice skin treated with MYCH-200, MYCH-400, MYCH-800, Commercial product and Oral solution at weeks 2, 4, 6 and 8.

DermaLab combo high-frequency ultrasound probe was used to measure dermal density and epidermal thickness at 2, 4, 6, and 8 weeks to evaluate the repairing capacity of different doses of MYCH and its oral solution on subacute aged skin (Figure 2b). The color of the ultrasound images corresponds to the density and thickness of the skin as follows: black < green < blue < red < yellow < white [30]. The dorsal skin of mice in the blank group consistently maintained high dermal density. In contrast, the skin in the model group exhibited low dermal density, indicating that oral administration of D-galactose leads to a decrease in skin dermal density. Compared to the model group, the dermal density in each experimental group showed a gradual increasing trend. At week 8, the dermal density in the oral solution group was close to that of the blank group. These results indicate that different doses of MYCH and its oral solution can repair subacute aged skin in mice, as evidenced by increased dermal density. The oral solution showed the most significant effect, followed by MYCH-800 and the commercial product. The superior effect of MYCH-800 over MYCH-200 and MYCH-400 is due to the positive correlation between the dose of MYCH and its efficacy. The oral solution exhibited the most significant effect because, in addition to MYCH, it also contains antioxidant ingredients such as vitamin C and hyaluronic acid, with vitamin C being a cofactor required for collagen synthesis.

Quantitative evaluation the capability of different doses of MYCH and its oral solution on subacute aged skin

The dermal density of the skin was measured using combo high-frequency ultrasound probe to evaluate the repairing capability of different doses of MYCH and its oral solution on subacute aged skin (Figure 3). The higher the value detected by the instrument, the better the condition of the skin [30]. The blank group consistently remained at a higher level, whereas the model group was relatively lower. By week 2, the dermal density in the MYCH-200 (59.18 ± 1.76), MYCH-400 (62.94 ± 1.94), MYCH-800 (69.14 ± 2.19), commercial product (66.63 ± 1.81), and oral solution groups (71.33 ± 1.93) were all lower than that of the blank group (85.75 ± 2.00), but higher than that of the model group (51.94 ± 1.91) (Figure 3a). By the 4th and 6th weeks, the dermal density in each experimental group continued to increase (Figure 3b, c). At week 8, the dermal density in the MYCH-200, MYCH-400, MYCH-800, commercial product, and oral solution groups increased to 72.07 ± 1.78, 77.02 ± 1.85, 82.16 ± 1.72, 81.09 ± 1.83, and 85.33 ± 1.86, respectively (Figure 3d). The dermal density in the oral solution group showed no significant difference from the blank group from the 6th week. These results indicate that different doses of MYCH and its oral solution contribute to restoring dermal density in subacute aged skin. The repair effect increases gradually with higher doses of MYCH. Additionally, the repair effect of MYCH-800 was superior to that of the commercial product group and slightly lower than that of the oral solution group. This is because the oral solution contains additional substances such as vitamin C and hyaluronic acid, which have antioxidant effects and promote collagen synthesis and cross-linking in the body.

The DermaLab combo moisture probe was used to measure skin hydration levels to study the efficacy of MYCH and its oral solution in repairing subacute aged skin (Figure 3e–h). When the skin is in good condition, its stratum corneum hydration level is usually higher, resulting in higher values detected by the instrument [31]. In week 2, the skin hydration levels of the model, MYCH-200, MYCH-400, MYCH-800, commercial product, and oral solution groups were all lower than that of the blank group (346.11 ± 21.35 μS), measuring 166.28 ± 21.60 μS, 175.48 ± 18.46 μS, 183.80 ± 19.84 μS, 204.83 ± 18.25 μS, 195.39 ± 19.67 μS, and 220.38 ± 18.90 μS, respectively (Figure 3e). At week 4, the skin hydration levels in the MYCH-200, MYCH-400, and MYCH-800 groups increased to 210.27 ± 18.32 μS, 230.55 ± 19.46 μS, and 254.18 ± 19.63 μS, respectively. The skin hydration levels in the commercial product and oral solution groups also increased to 246.08 ± 18.36 μS and 272.85 ± 18.14 μS, respectively, with a significant difference observed between these two groups (Figure 3f). At week 8, the skin hydration levels in the MYCH-200, MYCH-400, MYCH-800, commercial product, and oral solution groups increased to 246.20 ± 16.58 μS, 285.32 ± 19.29 μS, 305.76 ± 18.71 μS, 296.50 ± 19.45 μS, and 326.10 ± 19.02 μS, respectively (Figure 3g, h). The results indicate that MYCH and its oral solution contribute to the restoration of hydration levels in subacute aged skin. This is attributed to the oral absorption of MYCH, which enters the skin through the bloodstream, thereby increasing the levels of moisturizing factors like serine in the skin's stratum corneum and consequently enhancing skin hydration [32].

The TEWL probe was used to evaluate the epidermal water loss of mouse dorsal skin (Figure 3i–l). TEWL refers to the rate of moisture evaporation from the dermis through the epidermis, with higher values indicating greater moisture loss and poorer skin barrier function [33–34]. The TEWL values in the blank group remained at relatively low levels, while the TEWL values of the skin in the model group gradually increased. In the 2, 4, and 6 weeks, the TEWL values in each experimental group showed a gradual decrease trend (Figure 3i, j, k). By week 8, the TEWL values in the MYCH-200 (44.54 ± 1.79 g/m^2h^{-1}), MYCH-400 (42.54 ± 1.76 g/m^2h^{-1}), MYCH-800 (39.88 ± 2.01 g/m^2h^{-1}), commercial product (41.35 ± 1.98 g/m^2h^{-1}), and oral solution (38.33 ± 1.57 g/m^2h^{-1}) groups were significantly lower than those in the model group (67.31 ± 0.95 g/m^2h^{-1}). Importantly, the TEWL of the oral solution group showed no significant difference compared to the blank group (38.77 ± 1.66 g/m^2h^{-1}) (Figure 3l). The results indicate that oral administration of different doses of MYCH and its oral solution can repair the skin barrier of subacute aged skin, thereby restoring TEWL.

Histological evaluation of different doses of MYCH on the repair of subacute aged skin

Hematoxylin and eosin (H&E) staining was conducted to assess the ability of different doses of MYCH and its oral solution to repair subacute aged skin (Figure 4). The mice skin in the normal group consistently exhibited a healthy state. The epidermal layer was uniform and thin, the dermal-epidermal junction (DEJ) displayed undulating waves, and the collagen fibers in the dermis were full, densely intertwined, and well-organized, with no inflammatory cell infiltration. In contrast, the skin in the model group showed varying thicknesses of the epidermal layer, a flattened DEJ area, indistinct dermal papillae, and signs of inflammatory cell infiltration and vascular dilation. By week 2, the inflammatory cell infiltration in the dermis in each experimental group was slight reduction. In weeks 4 and 6, inflammatory responses and vascular dilation in the dermis gradually disappeared. At week 8, the DEJ area of the skin in each experimental group exhibited undulating waves again, with the skin in the oral solution group restored to normal level. These results indicate different doses of MYCH and its oral solution can improve the histological changes in aged skin. As the dose of MYCH increases, its repair effectiveness improves.

Masson's trichrome staining was used to explore the matrix remodelling effects of different doses of MYCH and its oral solution (Figure 5). The dermal collagen fibers in the blank group were densely and orderly arranged, presenting a wavy pattern. In contrast, the dermal collagen fibers in the model group showed a significant reduction, with signs of fragmentation and clustering observed. By week 2, new collagen fibers began to form at the sites of fracture and fragmentation in each experimental group, showing a relatively loose and disorganized arrangement. In weeks 4 and 6, there was a trend towards more organized alignment of dermal collagen fibers in each experimental group. At week 8, the dermal collagen fibers in each experimental group were repaired and exhibited a wavy pattern, particularly in the oral solution group where the dermal collagen fibers were restored to a nearly normal state. These results indicate that different doses of MYCH and its oral solution promote the regeneration and repair of collagen fibers in the dermal layer of subacute aged skin. As the dose of MYCH increases, its effectiveness improves. Additionally, oral products prepared with MYCH show superior effects compared to commercially available products.

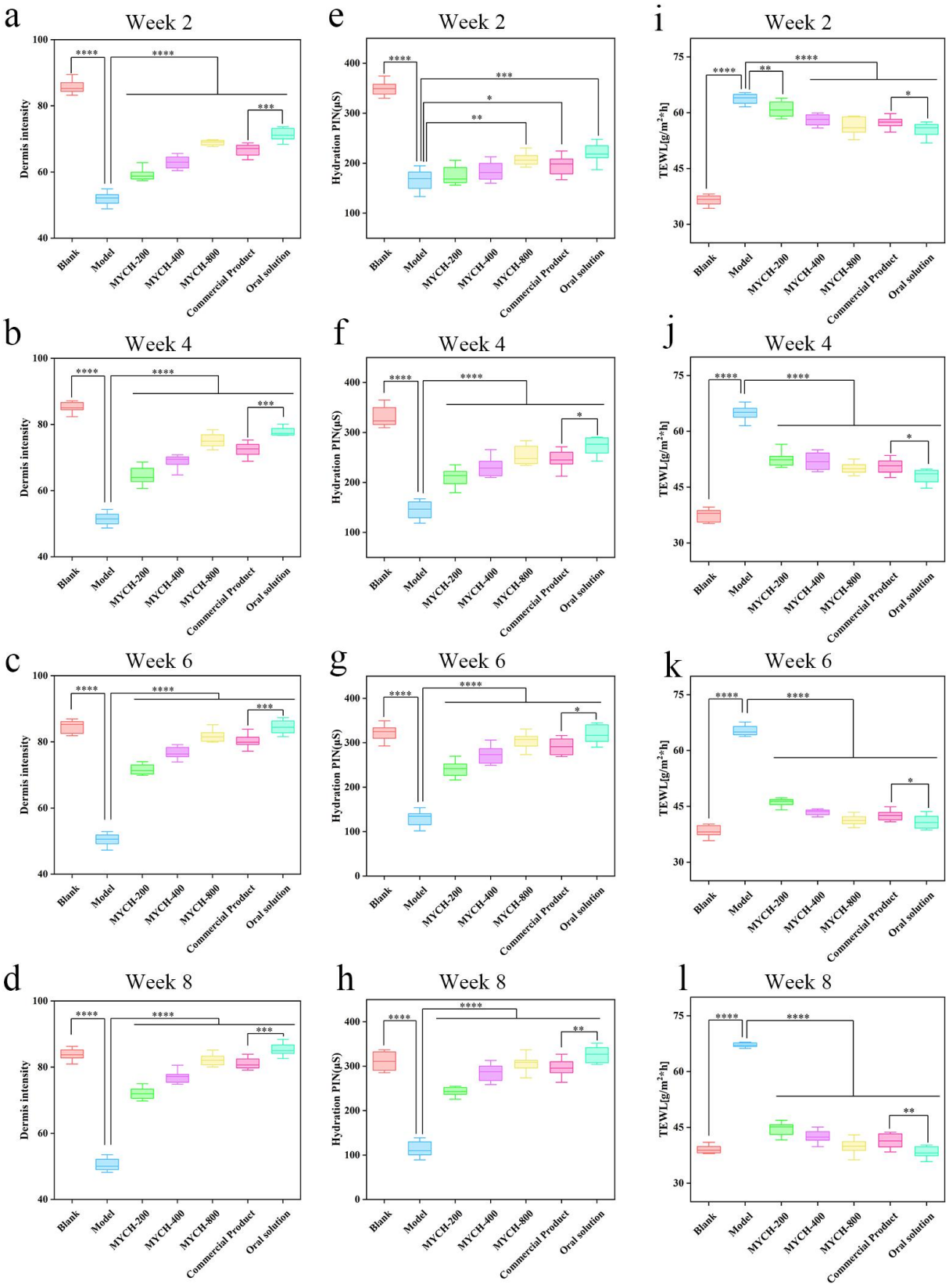

Figure 3 DermaLab combo quantitative evaluation of different doses of MYCH and its oral solution to repair subacute aging mouse skin. Dermal density (a–d), water content (e–h) and TEWL (i–l) of skin treated with MYCH-200, MYCH-400, MYCH-800 and Oral solution at weeks 2, 4, 6 and 8. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns > 0.05, n = 6.

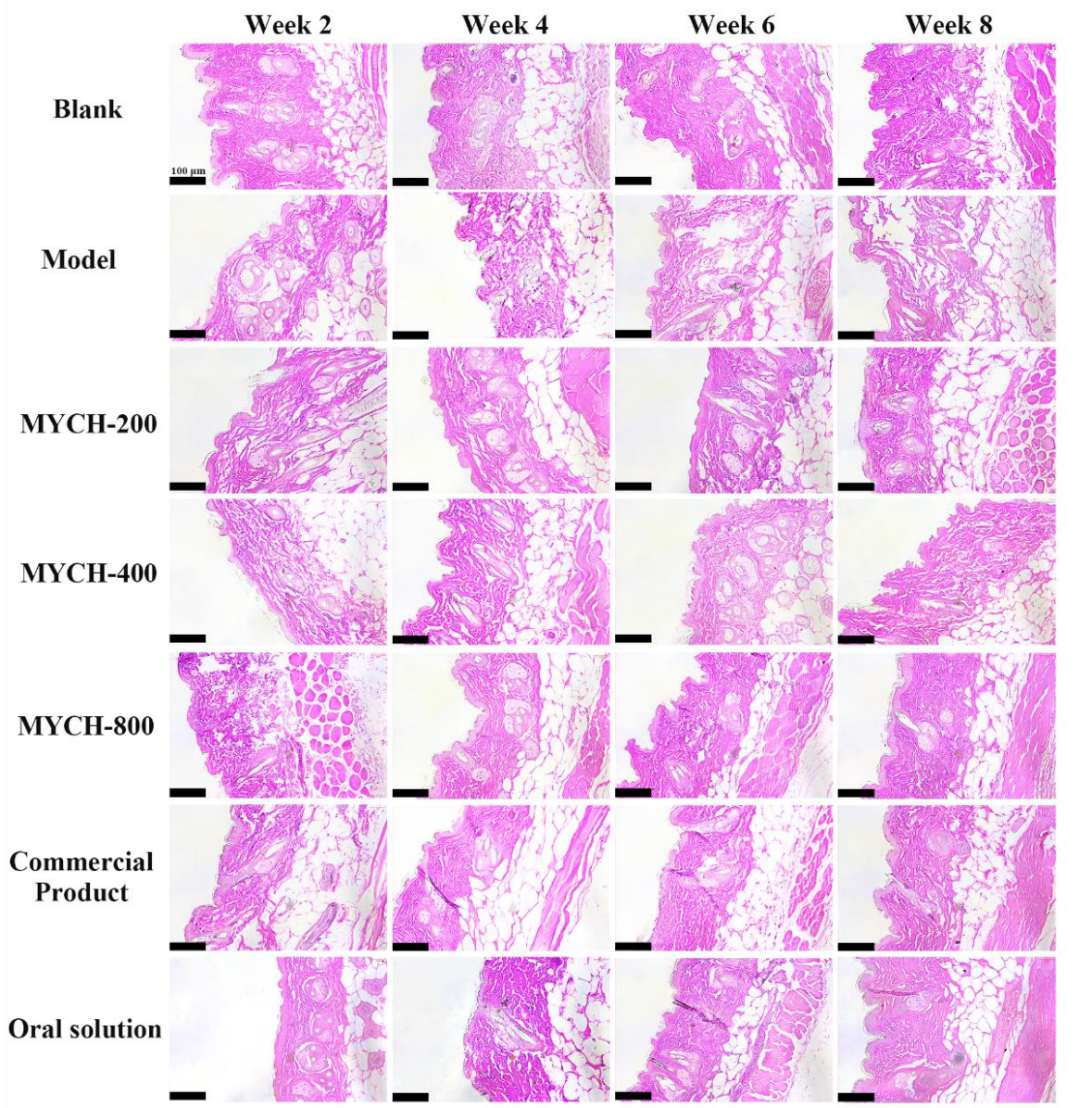

Figure 4 H&E staining analysis the performance of different doses of MYCH on repairing subacute aged skin. Scale bar: 100 μm.

Histological quantitative evaluation the effect of MYCH and its oral solution on the repair of subacute aged skin

Collagen fiber content in skin tissues at weeks 2, 4, 6, and 8 was detected to investigate the reparative capability of MYCH and its oral solution on subacute aged skin using Image J software (Figure 6a). At week 2, the collagen fiber content of MYCH-200, MYCH-400, MYCH-800, commercial product, and oral solution groups were all lower than that of the blank group (31.44 ± 1.64%), but higher than the model group (19.07 ± 1.67%), measured as 21.05 ± 1.71%, 22.60 ± 1.48%, 23.50 ± 1.03%, 22.94 ± 1.15%, and 24.82 ± 1.66%, respectively. By weeks 4 and 6, the collagen fiber content in the skin tissues of each experimental group gradually increased. By week 6, there was no significant difference in collagen fiber content between the homemade oral solution group (31.92 ± 1.43%) and the normal group (31.07 ± 1.79%). At week 8, the collagen fiber content in the skin tissues of the MYCH-200, MYCH-400, MYCH-800, commercial product, and oral solution groups increased to 25.73 ± 1.71%, 27.90 ± 1.62%, 29.73 ± 1.21%, 27.13 ± 1.41%, and 32.01 ± 1.61%.

Using the chloramine T method, the relative content of hydroxyproline (Hyp) in the dorsal skin tissue of mice in different groups was further quantitatively analyzed to evaluate the reparative ability of different doses of MYCH and its oral solution on subacute aged skin (Figure 6b). At week 2, relative to the Hyp content of the blank group (100%), the Hyp content in the model, MYCH-200, MYCH-400, MYCH-800, commercial product, and homemade oral solution groups decreased to 72.90 ± 3.60%, 74.26 ± 3.10%, 75.12 ± 2.26%, 76.00 ± 2.88%, 75.70 ± 2.77%, and 77.99 ± 2.93%, respectively. By weeks 4 and 6, the Hyp content in each experimental group gradually increased. By week 6, there was no significant difference in Hyp content between the solution group (96.40 ± 1.98%) and the blank group (99.66 ± 2.35%). By week 8, the Hyp content in the MYCH-200, MYCH-400, MYCH-800, commercial product, and oral solution groups was 83.00 ± 3.64%, 88.92 ± 2.98%, 95.14 ± 1.91%, 90.41 ± 2.94%, and 100.78 ± 2.33%, respectively. These results indicate different doses of MYCH and its oral solution can promote the collagen regeneration in subacute aged skin.

The SOD activity in the mice dorsal skin was measured to assess the in vivo antioxidant activity of MYCH at different doses and its oral solution (Figure 6c). SOD plays a critical antioxidant role in the skin, helping to eliminate free radicals and reduce oxidative stress reactions, thereby protecting skin cells from oxidative damage. Higher

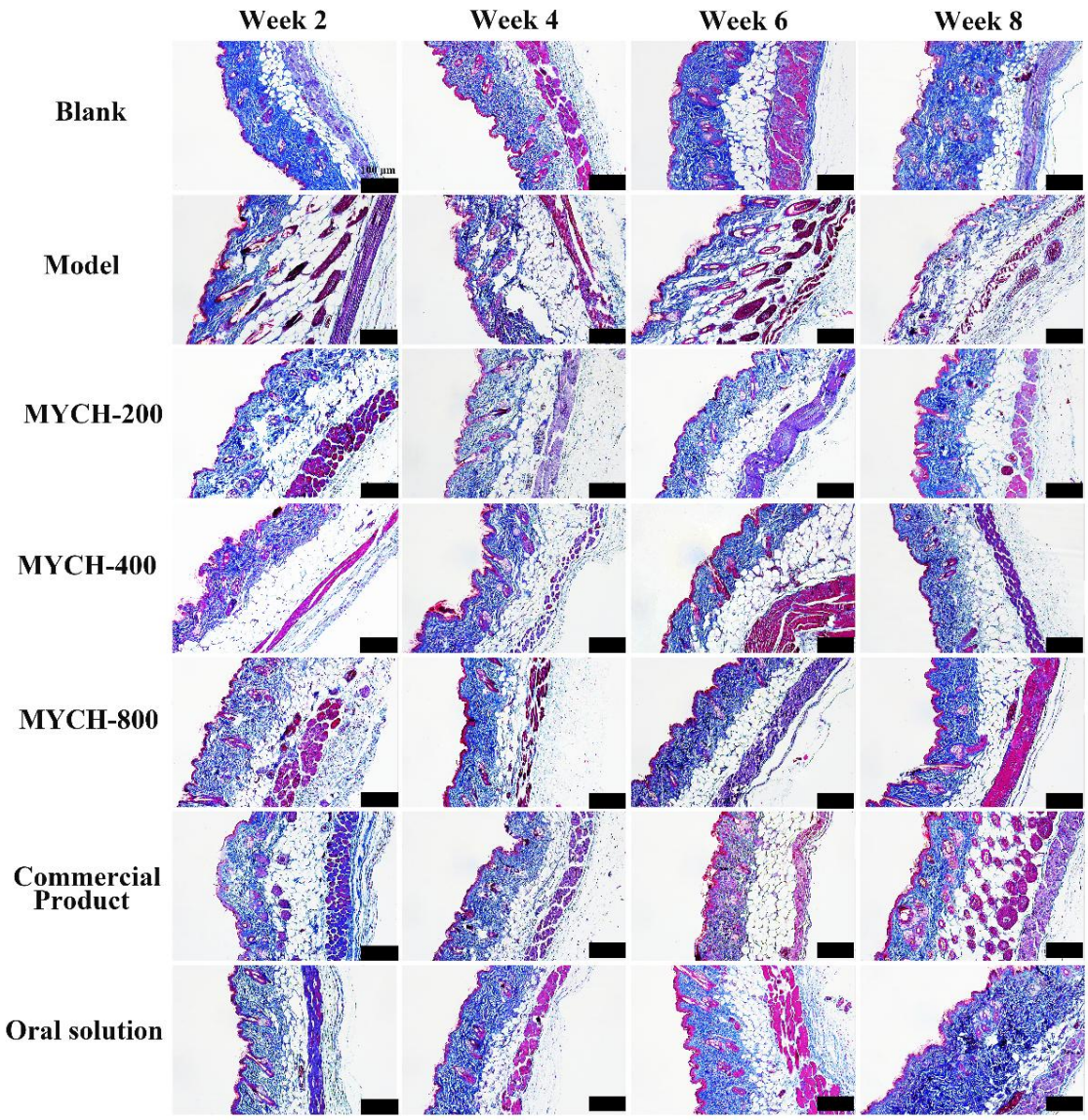

Figure 5 Masson staining evaluation the performance of different doses of MYCH on repairing subacute aged skin. Scale bar: 100 μm.

SOD values are detected when the skin is in better condition [35]. The mice skin in the blank group remained healthy, with SOD levels consistently maintained at high levels, whereas the model group consistently showed lower SOD levels. The SOD activity in each experimental group showed a gradual upward trend. At week 8, the SOD activity in the MYCH-200, MYCH-400, MYCH-800, commercial product, and oral solution groups increased to 139.58 ± 9.78 U/g, 162.23 ± 13.90 U/g, 175.98 ± 11.34 U/g, 166.38 ± 12.10 U/g, and 192.73 ± 10.11 U/g, respectively. Notably, the SOD activity in the oral solution group showed no significant difference compared to the normal group (197.97 ± 9.12 U/g), and significantly higher than that in the commercial product group. These results indicate that different doses of MYCH and its oral solution can enhance the activity of SOD in subacute aged skin tissues, thereby alleviating oxidative stress reactions. As the dose of MYCH increases, its antioxidant effect becomes stronger.

The malondialdehyde (MDA) content was used to further evaluate the in vivo antioxidant activity of MYCH at different doses and its oral solution (Figure 6d). MDA is a lipid oxidation product, and its increase in content is usually associated with oxidative stress and cell damage [1]. At week 2, the MDA content in the model, MYCH-200, MYCH-400, MYCH-800, commercial product, and oral solution groups was higher than that in the blank group (2.20 ± 0.17 nmol/g), increasing to 5.49 ± 0.25 nmol/g, 5.17 ± 0.29 nmol/g, 4.88 ± 0.15 nmol/g, 4.74 ± 0.19 nmol/g, 4.85 ± 0.27 nmol/g, and 4.74 ± 0.22 nmol/g, respectively. At weeks 4 and 6, the MDA content in each experimental group gradually decreased. At week 8, the MDA content in the MYCH-200, MYCH-400, MYCH-800, commercial product, and oral solution groups was 3.09 ± 0.13 nmol/g, 2.95 ± 0.24 nmol/g, 2.66 ± 0.21 nmol/g, 2.91 ± 0.09 nmol/g, and 2.52 ± 0.22 nmol/g, respectively. The MDA content in the oral solution group showed no significant difference compared to the normal group (2.45 ± 0.19 nmol/g). The results indicated that different doses of MYCH and its oral solution can reduce MDA content in subacute aged skin tissues, alleviating oxidative stress reactions.

Effect of MYCH on the expression of ECM regeneration-associated genes

Extracellular matrix (ECM) homeostasis is critical for maintaining skin structure and function and is tightly regulated by the balance between matrix synthesis and degradation. Matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-12, play key roles in age-associated ECM degradation, including the proteolysis of elastin and collagen components. To further explore the potential molecular basis underlying the skin-protective effects of MYCH, the expression levels of MMP-2 and MMP-12 were evaluated in a cell differentiation model

(Figure 7). Both MYCH and MYCH oral liquid treatments significantly reduced the expression of MMP-2 and MMP-12 compared with the blank group. These findings indicated that MYCH, in both its material form and oral liquid formulation, can suppress the expression of ECM-degrading enzymes at the cellular level. This reduction in MMP-2 and MMP-12 expression suggests a potential role of MYCH in attenuating ECM degradation and maintaining matrix homeostasis, which is consistent with the structural and functional improvements observed in the in vivo experiments.

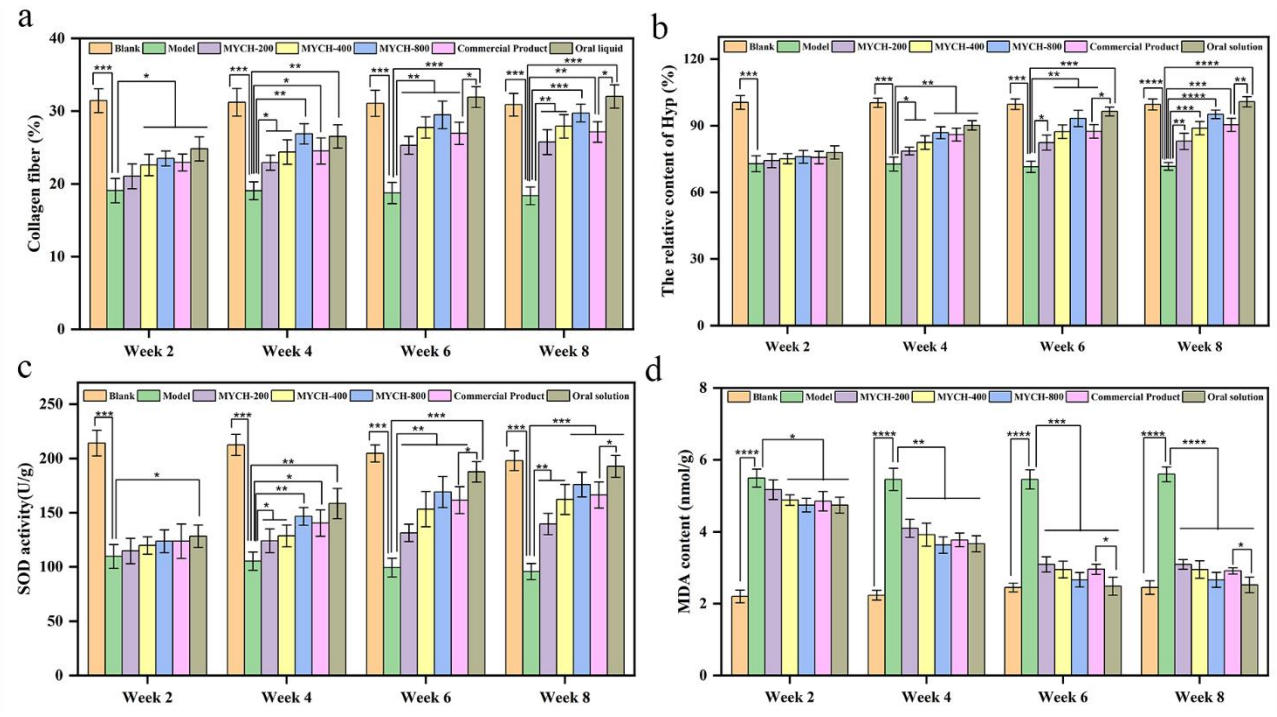

Figure 6 Histological analysis to evaluate the skin repair ability of different doses of medium molecular weight yak skin collagen hydrolysate and its oral solution. Collagen fiber (a), the relative content of Hyp (b), SOD activity(c), and MDA content (d) of mice skin on weeks 2, 4, 6 and 8. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns > 0.05, n = 3.

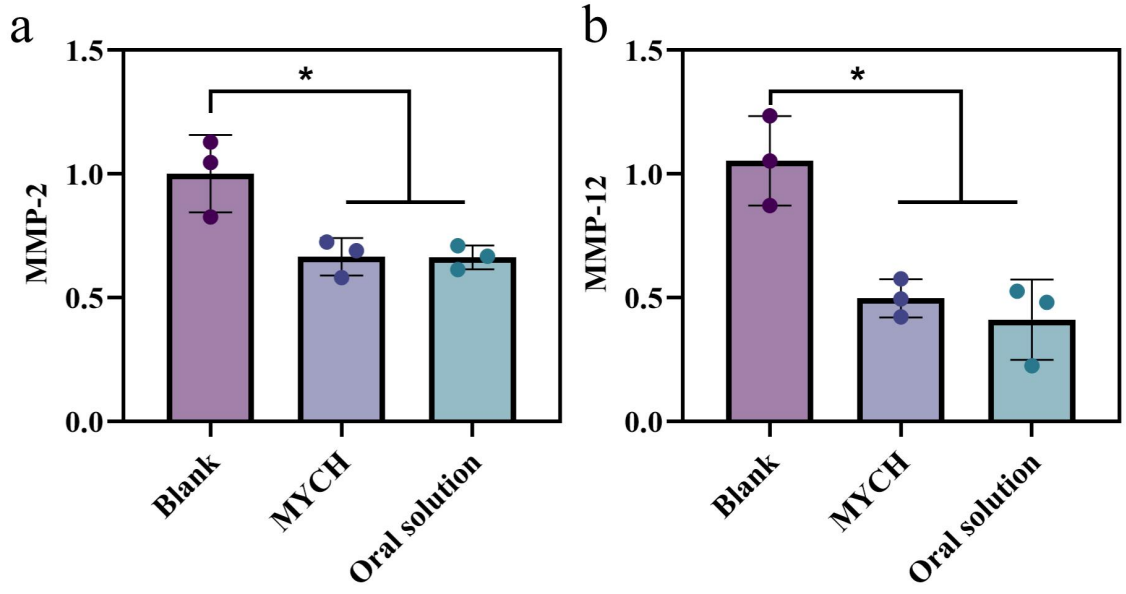

Figure 7 Relative gene expression analysis by RT-qPCR to evaluate the effects of MYCH and its oral solution on ECM regulation. mRNA expression levels of MMP-2 (a) and MMP-12 (b) following treatment with MYCH or oral solution. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns > 0.05, n = 3.

Conclusion

In this study, we evaluated the repair efficacy of different doses of medium molecular weight yak skin collagen hydrolysate (MYCH) on subacute aging mouse skin and developed an oral solution based on MYCH. DermaLab Combo assessment indicated that different doses of MYCH and its oral solution helped restore the density, moisture, and

TEWL of subacute aged skin to near-normal levels, with repair efficacy positively correlated with dosage. Histological analysis showed that MYCH and its oral solution improved pathological changes in subacute aged skin tissue, restored skin structure to normal levels, and significantly promoted collagen fiber regeneration. Antioxidant index analysis in skin tissue demonstrated that MYCH and its oral solution helped reduce oxidative stress induced by long-term D-galactose administration.

Furthermore, cell-based experiments showed that MYCH and its oral solution could inhibit the expression of ECM-degrading enzymes MMPs, supporting the maintenance of matrix homeostasis. The oral formulation was designed with reference to related commercially available products, combining MYCH with vitamin C, hyaluronic acid, bilberry extract, and highland plant ingredients. Although this study focused on biological efficacy, future work will address formulation stability, active ingredient retention, and safety to establish a robust quality control system. These findings suggested that MYCH-based oral products can effectively improve skin condition and have potential applications in nutritional supplements and skincare products.

This study demonstrates that oral administration of yak collagen peptides can effectively delay skin aging. However, the underlying mechanisms, particularly at the genomic level, have not yet been fully elucidated. Further investigation is required to uncover the specific molecular pathways and cellular processes through which collagen peptides exert their anti-aging effects. Future research will focus on exploring these mechanisms in depth, including comprehensive omics analyses, such as genomics, transcriptomics, and proteomics, as part of our ongoing commitment to advancing this field.

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