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Research Article

The Effect of a Collagen Peptide – Daily Glow® on the Improvement of Elasticity, Hydration, Redness and Roughness of Facial Skins: A Human Clinical Trial and the Mechanisms

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Abstract

In recent years, collagen peptides have been becoming the dominant products in the oral beauty market as various scientific researches have demonstrated they can stimulate the dermal fibroblasts that secrete various components such as collagen and elastin in the extracellular matrix. In our clinical study, 30 individuals were orally supplemented with a collagen peptide called Daily Glow® for 28 days, and the facial skin conditions including hydration, trans-epidermal water loss and elasticity were significantly and positively improved. Besides, it was clearly observed by the Antera 3D Miravex that redness and roughness were significantly improved. Our vitro test of human dermal fibroblast showed mRNA expression of MMP has been inhibited while mRNA expressions of LOX, FBN2 and AQP3 have been stimulated by Daily Glow®. This should be the first time that these mechanisms are newly proposed and demonstrated.

Introduction

Skin appearance can indicate the age and health state by showing wrinkles, unevenly facial colors and black spot [1]. The fragmentation of the collagen matrix in the dermis occurs in an aged skin due to various reasons such as UV exposure [2]. However, the fragmented collagen originally produced and organized in fibroblasts cannot be reconnected because the synthesis of collagen and other extracellular matrix proteins are regulated by fibroblasts in response to mechanical tension that never exists during fragmentation [3]. Without the stretch of the collagen fibers, the fibroblasts not only produce insufficient amounts of collagen but also generate high level of collagen-degrading enzymes [4]. Therefore, the strategy of boosting the collagen synthesis by stimulating the damaged fibroblasts and its proliferation is preferred. Recently various human clinical trials have shown that orally administered collagen peptide is potential and beneficial in repairing the aged skin by stimulating the fibroblasts and collagen synthesis [5,6]. The mechanism lies in specific configurations of the unique triple helix structure with a repeating amino acid sequence of (glycine–X–Y) in collagen peptides. For instance, the structure of prolyl-hydroxyproline might function as chemotactic stimuli for fibroblasts in vivo and attract these cells to the damaged sites and tissues for restoration [7]. Besides, prolyl-hydroxyproline could also enhance proliferation human dermal fibroblasts as well as hyaluronic acid synthesis in a vitro study [8].

On the other hand, however, more mechanisms for collagen peptides supplement remains to be investigated. Matrix metalloproteinases (MMPs), a family of matrix protein–degrading enzymes, initiate the breakdown of collagen fibers [9]. Under UV

exposure, the generation of reactive oxygen species lead to the overproduction of MMPs and subsequently photo-aging [10]. Various skin antioxidants are applied in order to scavenge those ROS [11]. In our study, we hypothesize that certain structures in the collagen peptides should be capable of inhibiting the activities of MMPs. Moreover, it should not be ignored that the transportation of water and glycerol in the basal layer keratinocytes of epidermis in normal skin cannot be finished without a membrane transporter called Aquaporin-3 (AQP3). It was found AQP3-knockout mice have reduced stratum corneum water content [12&13]. Here we also hypothesize the supplementation of collagen peptides could activate the expression of AQP3 and thus facilitate the hydration of epidermis. Therefore, in our investigation, not only did we conduct a human trial to demonstrate the effect a collagen peptide on the improvement of facial skin conditions, but also did we analyze the mechanisms of relative gene expressions in human dermal fibroblasts, which were contributed by the collagen peptide in a vitro study.

Materials and Methods

Testing product

Daily Glow® was provided by Zhejiang Xinmei Biotechnology Co., Ltd. Fruit powders were formulated with Daily Glow® to form a total of 10 g powder product with favored taste.

Effect of Daily Glow® on human dermal fibroblasts in vitro test

This test was carried out with the assistance from Shanghai Huiwen Biotech Corp., Ltd. The solution of collagen peptide was prepared at 10%, 5%, 0.5%, 0.1% solution with sterile PBS. The solution of EGF (GIBCO) was prepared at 10 ng/ml solution with sterile PBS. The Human dermal fibroblast cells were inoculated (0.4 ~ 1.6x105 cells/well) into the 6-well cell culture plate with collagen peptides. The RNA extraction and reverse transcription cDNA were operated by the RNA extraction kit and Prime Script™ RT Master Mix. The primer verification of RT-PCR and RT-PCR tests (Table 1) were conducted by TB green ® Premix Ex Taq™ II (Takara).

Table 1: Primer name and sequence of different genes in RT-PCT.

Primer Name	Primer Sequence
GAPDH-F	GAAATCCCATCACCATCTTCCAGG
GAPDH-R	GAGCCCCAGCCTTCTCCATG
AQP3-3F	GCAGCCTGTCCATCTGTG
AQP3-3R	ACCCTACTTCCCAAAAGCC
MMP1-1F	ACACGCCAGATTTGCCAAGAGC
MMP1-1R	GGAGAGTTGCCCGATGATCTCCCC
LOX-F	CCTACTACATCCAGGCGTCCA
LOX-R	CATAATCTCTGACATCTGCCCCTGT
FBN2-F	CCTACTACATCCAGGCGTCCA
FBN2-R	CATAATCTCTGACATCTGCCCCTGT

Intervention

The study was carried out as a single-blinded, randomized and self-controlled trial on the effects of 30 healthy male and female subjects after 14 and 28 days of oral intake. Thirty healthy female subjects in the age group of 27 to 39 years were screened and enrolled in the study. Twenty-five subjects completed the study and five withdrew due to personal reasons. They are capable of reading Chinese and comprehending the consent agreement; they are willing to cooperate and complete all the tests during the study and report any adverse events. The participants were supplemented with one sachet of Daily Glow® once a day. Before inception of the tests, all participants signed consent agreement that manifests their benefits from this test and relevant risks.

Exclusion Criteria

Females that are pregnant or breastfeeding; any obvious facial defects including sunburn, scar, pigmented nevus, which might impair the test characterization; facial microbial infections; chronic skin diseases (such as skin tumors, rosacea, eczema, lupus erythematosus, seborrheic dermatitis, psoriasis, severe epidermal shedding); history of immunosuppressive or immunodeficiency disorders (including HIV or AIDS) or current use of immunosuppressive drugs or radiotherapy; chronic and endocrine diseases such as asthma, epilepsy, diabetes, hypertension, hyperthyroidism or hypothyroidism; participation of other clinical studies during the past 3 months; took any drugs that may affect skin status or response in the past 6 months or currently such as antihistamines, antibiotics, insulin, anti-inflammatory drugs, vitamins A, steroids, aspirin, thyroid drug; treated with facial medical treatment, such as laser treatment, chemical stripping and minimally invasive cosmetic treatment; a history of mental illness or unable to take care of themselves; participant with any of the above were excluded from the study.

Outcome Measures

All measurements were performed in the same room with no

daylight under controlled ambient conditions (20°C-24°C, 48%-50% relative humidity). Each subject was required to clean the face 30 minutes before the test.

Measurement of Elasticity

The measuring principle of the Cutometer® MPA 580 (Figure 1) is based on the suction method, where negative pressure deforms the skin mechanically. Inside the probe, the penetration depth is determined by a non-contact optical measuring system. This optical measuring system consists of a light source and a light receptor, as well as two prisms facing each other, which project the light from transmitter to receptor. The light intensity varies due to the penetration depth of the skin. The resistance of the skin to the negative pressure (firmness) and its ability to return into its original position (elasticity) are displayed as curves (penetration depth in mm/time) in real time during the measurement. From these curves, parameters related to elastic and viscoelastic properties of skin surface can be calculated. The closer the value gets to 1, the more elastic the skin is.

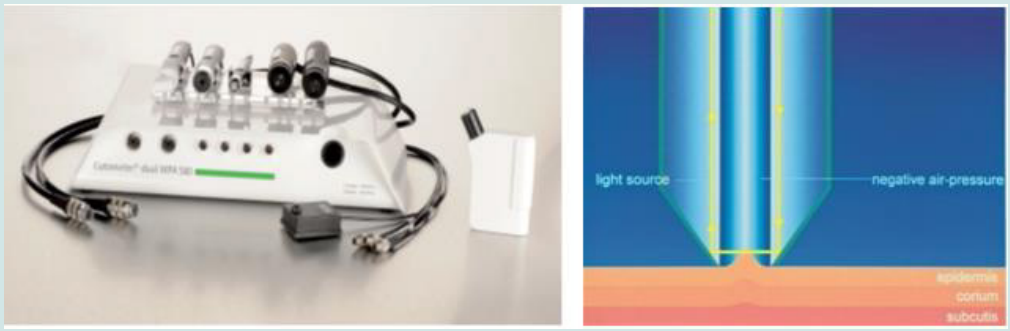

Figure 1: Cutometer® MPA580.

Measurement of Hydration at Skin Surface

The hydration level off the skin surface (stratum corneum) was characterized by Corneometer®, Derma Unit SSC 3 (Figure 2). The probe allows very quick measurement (1 s) that is crucial to avoid any Occlusion. Substances on the skin such salts or residues of topical applied products have only minimal impact due to capacitance measurement. Larger value reflects higher extent of hydration at skin surface.

Figure 2: Craniometer®, Derma Unit SSC 3.

Trans-epidermal Water Loss and Skin Barrier Function

The Terameter® TM 300 with its "open chamber" principle is worldwide accepted measuring device for the assessment of the trans-epidermal water loss (TEWL). This is an indispensable parameter for the evaluation of the water barrier function of the skin and a basic measurement in all kinds of applications. Even the slightest damage in the skin water barrier can be determined at an early stage (Figure 3). The Antera 3D is a camera for image acquisition and analysis of the skin, which relies on multi-directional illumination and computer-aided reconstruction of the skin surface from different angles. The skin topography and the chromophores' concentration are derived from the spatial and spectral analysis of the acquired image data from skin illumination (Figure 4).

Figure 3: Terameter® TM 300.

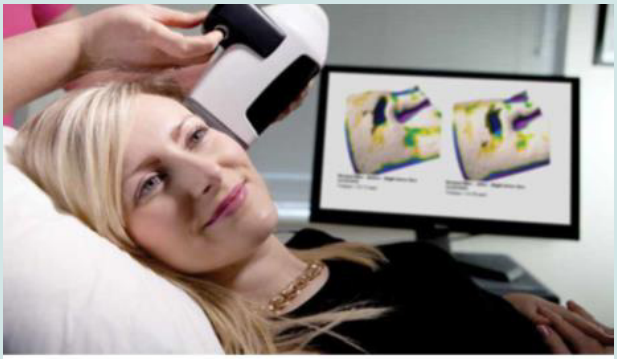

Figure 4: Antera 3D.

Adverse Events

The safety and tolerability of the administration of treatment powder were evaluated at day 14 and day 28. Professional dermatologist asked the subjects whether they had gastrointestinal discomfort, body skin tingling, itching and other symptoms or obvious signs of dry skin, desquamation, flushing and so on. During the whole study, the participants were requested to report instantly to the investigator once they experienced any uncomfortable feelings.

Statistical Analysis

Statistical analyses of this clinical study were completed using IBM Statistical Package for Social Sciences (SPSS 21.0) at an alpha level of 0.05. To evaluate primary and secondary outcome measures, analysis of variance (ANOVA) was used to compare within-group changes and group changes over time.

Results

Table 2: Effect of Daily Glow® on gene expressions of fibroblast cells in vitro.

	AQP3	MMP1	FBN2	LOX
Control	100.00%	100.00%	100.00%	100.00%
EGF	175.50%	36.97%	103.82%	382.28%
1	581.28%	25.72%	44.74%	482.69%
0.5	86.26%	38.45%	225.09%	360.31%
0.05	215.48%	73.22%	231.90%	519.56%
0.01	197.69%	151.56%	223.44%	490.19%

Table 3: Effect of Daily Glow® on hydration in the corneum.

Time (day)	Hydration in the Corneum	Trans Epidermal Water Loss
0	51.79±2.93	20.18±1.71
14	57.41±3.12**	17.28±1.63**
28	64.41±2.66**	15.15±1.38**

Table 4: Effect of Daily Glow® on skin elasticity, roughness and redness.

Time (day)	Skin Elasticity	Skin Roughness	Skin Redness
0	0.53±0.02	18.00±2.22	35.00±3.25
14	0.62±0.03**	14.00±1.89**	30.00±3.15**
28	0.69±0.02**	13.20±1.67**	27.00±2.63**

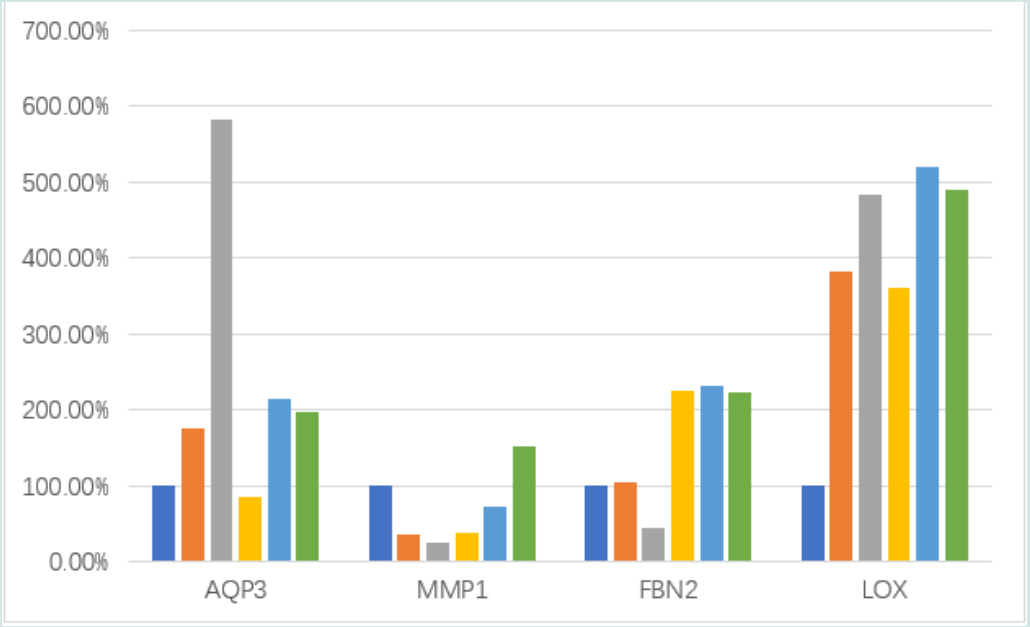

Figure 5: Effect of Daily Glow® on gene expressions of fibroblast cells in vitro.

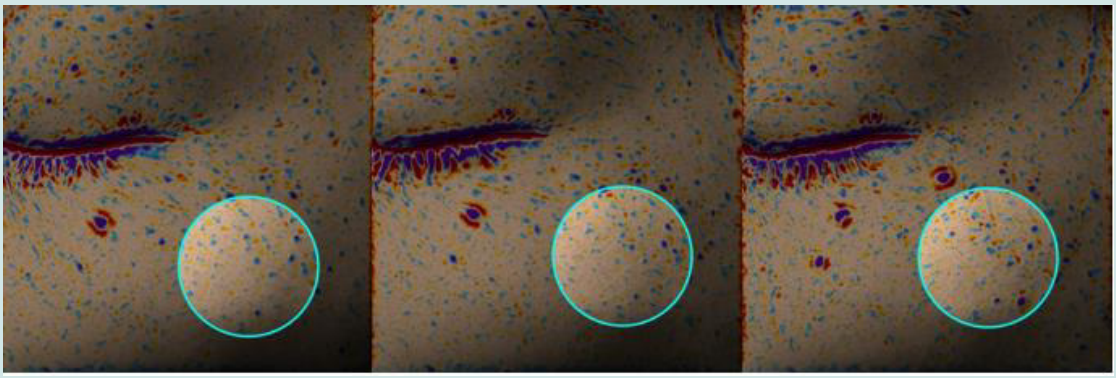

Figure 6: The fine lines and wrinkles of a subject at Day 0, 14 and 28 of Daily Glow® supplementation.

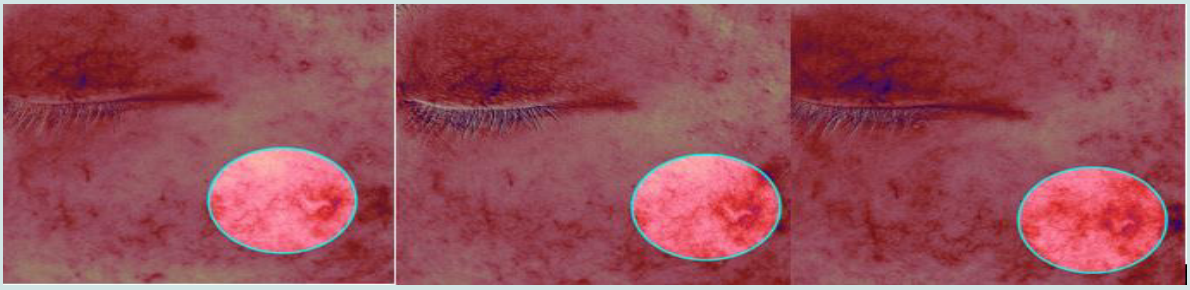

Figure 7: The skin redness of a subject at Day 0, 14 and 28 of Daily Glow® supplementation.

To investigate the effect of the collagen peptide on fibroblasts, various mRNA expressions are shown in (Table 2) and (Figure 5). The mRNA expression of AQP3 was increased when the concentration of collagen peptide increased from 0.01 to 1. The mRNA expressions of FBN2 and LOX were larger than those stimulated by EGF while they did not manifest in a dose-dependent manner. Besides, the mRNA expression of MMP1 was reduced as the concentrations of collagen peptide increased. It is not difficult to see at certain levels the effects of collagen peptides were comparable and even superior to those generated by EGF. The significant improvement of hydration, trans-epidermal water loss, elasticity, roughness and redness by supplementing Daily Glow® for 14 and 28 days are shown in (Tables 3&4). The significant improvement of fine lines, wrinkles and redness of the subjects' facial skin after supplementing Daily Glow® for 14 and 28 are shown in (Figures 6&7).

Discussion

There have been many animal and clinical research advances in the protection and improvement of skin by collagen peptides regarding skin elasticity, hydration, trans-epidermal water loss, photo aging, atopic skin and so on [14-16]. In our human trial, we observed the similar positive effects of collagen peptide administered to healthy subjects for 28 days. The skin conditions were improved from perspectives of skin hydration in the corneum, trans-epidermal water loss, skin elasticity, skin redness and skin roughness by professional instrumental characterization of facial skins. Collagen peptides can repair and increase the activity of fibroblasts and thus increase the production of hyaluronic acid and collagen fibrils [17]. In particular collagen tripeptides and di-peptides can stimulate the activity of fibroblasts with lost mechanical strength [18,19]. In our study, we investigated profoundly in the mechanisms of such effects. Firstly, under UVA exposure, a great amount of reactive oxygen species (ROS) would be generated under the skin, consequently increasing the production of matrix metalloproteinases (MMP), which is a group of protease enzymes responsible for the degradation of the extracellular matrix including collagen and elastin fibers [20]. In our vitro study, the mRNA expression of MMP1 was reduced when fibroblasts were co-cultured with Daily Glow®. As antioxidants could effectively scavenge ROS, formulas that contain both collagen peptide and antioxidants (e.g., plant-based SOD) could protect skin from a cascade of UV-exposed reactions [21]. In the futuristic research, the specific structure of the oligopeptides should be characterized and standardized regarding free radical scavenging capacities.

On the other hand, accumulating evidence indicates that the water-, glycerol- and hydrogen peroxide-transporting channel aquaporin-3 (AQP3) expressed in plasm membranes in a variety of cells plays a key role in various processes involved in keratinocyte function while abnormalities in this channel have been observed in several human skin diseases [22]. Hydration of the SC is an important determinant of skin appearance and highly depends on AQP3. Exposure of mice to high humidity or skin occlusion increased SC hydration in the wild type, but not in AQP3-null mice [23]. Also, it was found the magnitude of water moving into SC to off-set evaporative water is much lower than that with AQP3 [24]. In our research, the increased mRNA expression of AQP3 probably indicates the improvement in the SC hydration and trans-epidermal water loss. Both our vitro study and literature show certain structure in the collagen peptide should be similar with epidermal growth factor; however, this remains to be investigated in the futuristic studies [25]. One of the most significant components in extracellular matrix, elastic fibers are composed of elastin and microfibrils mainly structured with fibrillin (large cysteine-rich glycoproteins), which are encoded by distinct genes such as FBN2 [26]. Also, elastic fiber formation cannot be realized without lysyl oxidase (LOX), which plays a critical role in the catalysis of lysine-derived crosslinks of elastin in the dermal extracellular matrix. In our vitro study, the mRNA expressions of FBN2 and LOX have been enhanced in human dermal fibroblasts stimulated by Daily Glow®. In general, our clinical study showed the administration of Daily Glow® to healthy subjects for 28 days significantly improved facial skin conditions in terms of elasticity, hydration, redness and so on. As various research has demonstrated the stimulation of collagen peptides on dermal fibroblasts, our vitro study should be the first scientific report that has verified the activation of several genes expressions by collagen peptides, which are extremely crucial for the fibroblasts to repair the skin.

References

1. Yaar M, Eller MS, Gilchrest BA (2002) Fifty years of skin aging. The journal of investigative dermatology. Symposium proceedings 7(1): 51-58.
2. Haratake A, Watase D, Fujita T, Setoguchi S, Matsunaga K, et al. (2015) Effects of oral administration of collagen peptides on skin collagen content and its underlying mechanism using a newly developed low collagen skin mice model. Journal of Functional Foods 16: 174-182.
3. Fisher GJ, Varani J, Voorhees JJ (2008) Looking older: fibroblast collapse and therapeutic implications. Archives of dermatology 144(5): 666- 672.
4. Varani J, Dame MK, Rittie L, Fligiel SE, Kang S, et al. (2006) Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. The American journal of pathology168(6):1861-1868.
5. Proksch E, Schunck M, Zague V, Segger D, Degwert J, et al. (2014) Oral intake of specific bioactive collagen peptides reduces skin wrinkles and increases dermal matrix synthesis. Skin pharmacology and physiology 27(3): 113-119.
6. Proksch E, Segger D, Degwert J, Schunck M, Zague V, et al. (2014) Oral supplementation of specific collagen peptides has beneficial effects on human skin physiology: a double-blind, placebo-controlled study. Skin pharmacology and physiology 27(1): 47-55.
7. Postlethwaite AE, Seyer JM, Kang AH (1978) Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides. Proceedings of the National Academy of Sciences of the United States of America 75(2): 871-875.
8. Ohara H, Ichikawa S, Matsumoto H, Akiyama M, Fujimoto, et al. (2010) Collagen-derived dipeptide, proline-hydroxyproline, stimulates cell proliferation and hyaluronic acid synthesis in cultured human dermal fibroblasts. The Journal of dermatology 37(4): 330-338.
9. Lapière C (2005) Tadpole collagenase, the single parent of such a large family. Biochimie 87(3-4): 243-247.

10. Baek B, Lee SH, Kim K, Lim HW, Lim CJ (2016) Ellagic acid plays a protective role against UV-B-induced oxidative stress by up-regulating antioxidant components in human dermal fibroblasts. The Korean journal of physiology & pharmacology : official journal of the Korean Physiological Society and the Korean Society of Pharmacology 20(3): 269-277.

11. Egoumenides L, Gauthier A, Barial S, Saby M, Orechenkoff C, et al. (2018) A Specific Melon Concentrate Exhibits Photoprotective Effects from Antioxidant Activity in Healthy Adults. Nutrients 10(4): 437.

12. Kim DU, Chung HC, Choi J, Sakai Y, Lee BY (2018) Oral Intake of Low-Molecular-Weight Collagen Peptide Improves Hydration, Elasticity, and Wrinkling in Human Skin: A Randomized, Double-Blind, Placebo-Controlled Study. Nutrients 10(7): 826.

13. Hara-Chikuma M, Verkman AS (2008) Roles of aquaporin-3 in the epidermis. The Journal of investigative dermatology 128(9): 2145-2151.

14. Choi SY, Ko EJ, Lee YH, Kim BG, Shin HJ, et al. (2014) Effects of collagen tripeptide supplement on skin properties: a prospective, randomized, controlled study. Journal of cosmetic and laser therapy : official publication of the European Society for Laser Dermatology 16(3): 132-137.

15. Pyun HB, Kim M, Park J, Sakai Y, Numata N, et al. (2012) Effects of Collagen Tripeptide Supplement on Photoaging and Epidermal Skin Barrier in UVB-exposed Hairless Mice. Preventive nutrition and food science 17(4): 245-253.

16. Berardesca E, Abril E, Serio M, Cameli N (2009) Effects of topical gluco-oligosaccharide and collagen tripeptide F in the treatment of sensitive atopic skin. International journal of cosmetic science 31(4): 271-277.

17. Fisher GJ, Varani , Voorhees JJ (2008) Looking Older. Archives of Dermatology 144(5): 666-672.

18. Sontakke SB, Jung J, Piao Z, Chung, HJ (2016) Orally Available Collagen Tripeptide: Enzymatic Stability, Intestinal Permeability, and Absorption of Gly-Pro-Hyp and Pro-Hyp. Journal of Agricultural and Food Chemistry 64(38): 7127-7133.

19. Cadet J, Douki T, Ravanat, JL (2015) Oxidatively generated damage to cellular DNA by UVB and UVA radiation. Photochemistry and photobiology 91(1): 140-155.

20. Egoumenides L, Gauthier A, Barial S, Saby M, Orechenkoff C, at al. (2018) A Specific Melon Concentrate Exhibits Photoprotective Effects from Antioxidant Activity in Healthy Adults. Nutrients 10(4): 437.

21. Bollag WB, Aitkens L, White J, Hyndman KA (2020) Aquaporin-3 in the epidermis: more than skin deep. American journal of physiology. Cell physiology 318(6): C1144-C1153.

22. Hara M, Ma T, Verkman AS (2002) Selectively reduced glycerol in skin of aquaporin-3-deficient mice may account for impaired skin hydration, elasticity, and barrier recovery. The Journal of biological chemistry 277(48): 46616-46621.

23. Hara M, Verkman AS (2003) Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice. Proceedings of the National Academy of Sciences of the United States of America 100(12): 7360-7365.

24. Cao C, Sun Y, Healey S, Bi Z, Hu G, et al. (2006) EGFR-mediated expression of aquaporin-3 is involved in human skin fibroblast migration. The Biochemical journal 400(2) 225-234.

25. Uehara E, Hokazono H, Hida M, Sasaki T, Yoshioka H, et al. GABA promotes elastin synthesis and elastin fiber formation in normal human dermal fibroblasts (HDFs). Bioscience, biotechnology, and biochemistry 81(6): 1198-1205.

26. Szauter KM, Cao T, Boyd CD, Csiszar K (2005) Lysyl oxidase in development, aging and pathologies of the skin. Pathologie-biologie 53(7): 448-456.

DOI: 10.32474/CTBM.2022.03.000154