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Evaluation of the anti-aging effects of Zinc-α2-glycoprotein peptide in clinical and in vitro study

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Abstract

Background: Skin aging, characterized by the deterioration of skin density and elasticity, is a common concern among individuals seeking to maintain a youthful appearance. Zinc-α2-glycoprotein (ZAG) is secreted by various body fluids, and is associated with lipolysis and identified as an atopic dermatitis biomarker. This study evaluated the potential of ZAG peptides, which exert multiple benefits such as anti-aging.

Materials and Methods: We conducted a 4-week clinical trial on patients with noticeable periorbital wrinkles ($n = 22$) using a ZAG peptide-containing product. The effects of the products on skin density, elasticity, and the depth of periorbital wrinkles were evaluated using Cutometer Dual MPA580, Ultrascan, and Antera 3D CS, respectively. The effect of ZAG peptides on UVB-treated keratinocyte cells was evaluated in vitro to understand the mechanisms underlying its effects against impaired skin barrier function, collagen degradation, and senescence. In addition, the effects of ZAG peptides on cell viability and expression of aging and skin barrier-related genes were assessed using cell counting kit assay and quantitative reverse transcription-polymerase chain reaction, respectively.

Results: The patients demonstrated improved skin density, elasticity, and reduced periorbital wrinkles. Further, more than 85% patients scored the product as satisfactory regarding anti-aging effects. Furthermore, ZAG peptides reduced SA-β-gal staining, downregulated the senescence-related genes, and upregulated the skin barrier function-related genes in UVB-irradiated keratinocyte cells.

Conclusions: Our clinical and in vitro findings showed that ZAG peptides exert anti-aging effects and improve skin barrier functions, suggesting their promising potential as therapeutic agents to combat skin aging and improve skin health.

KEYWORDS
anti-aging, senescence-related genes, skin barrier, skin physiology, skin structure, UVB irradiation, ZAG peptide

1 | INTRODUCTION

Skin aging is a complex and multifactorial process influenced by both intrinsic and extrinsic factors.[1] As individuals age, their skin undergoes various changes, such as wrinkles and dryness, due to the progressive loss of collagen, elastin, and hyaluronic acid.[2–4] The combination of intrinsic and extrinsic factors leads to gradual and inevitable changes in skin appearance associated with aging.[1] Over time, the skin becomes thinner, less elastic, and more prone to injury. Environmental factors, such as sun exposure, smoking, and other lifestyle behaviors, also affect skin aging.[4] Therefore, preventing or slowing down the aging process caused by these factors is crucial.

The skin barrier is essential in maintaining skin hydration and protecting the skin from environmental damage.[5] During aging, the skin's natural substances, such as collagen, elastin, and hyaluronic acid, decrease, weakening skin barriers.[3] The decline in skin barrier function accelerates skin aging by reducing the skin's ability to protect itself from environmental stressors. The vicious cycle of skin aging and skin barrier damage is exacerbated by unprotected exposure to environmental pollutants, UV radiation, and harsh cleansers, which increase oxidative stress and accelerate skin aging.[6] Therefore, using products and practices that can strengthen and restore the skin barrier is crucial to delay the signs of premature aging.

ZAG, a multidisciplinary protein, has been detected in various human body fluids and epithelial cells of the prostate, liver, and breast.[7,8] In a previous study, ZAG treatment in an atopic dermatitis-induced mouse model enhanced the mRNA expression of filaggrin (*Flg*). It reduced the mRNA expression of genes associated with transepidermal water loss (TEWL) and inflammatory factors.[9] Moreover, *ZAG* mRNA degradation via shRNA in normal human epidermal keratinocytes has also been shown to reduce *FLG* expression, and the ZAG peptide has been shown to have multiple effects on skin health.[9] Despite the demonstrated stability and other beneficial effects of the ZAG peptide, detailed studies on its anti-aging effects on the skin are lacking. Hence, this study aimed to investigate the anti-aging effects of the ZAG peptide and products containing it on skin health.

2 | MATERIALS AND METHODS

2.1 | Participants

Twenty-two female patients (35—60 years old) with noticeable periorbital wrinkles were enrolled in this study. Participants with a history of dermatologic treatments (e.g., fillers, scaling, tattoos, Botox, and laser), recent use of similar products to those used in this study within 3 months, skin lesions, allergies or hypersensitivity, infectious skin diseases, and adverse responses to medications, cosmetics, or routine light exposure were excluded. All participants were instructed to apply Ato Repair Cleaner, Ato Repair Lotion, and Ato Repair Cream (Ato Repair Solutions containing ZAG peptides; L&C Bio Inc., Seoul, Korea) twice daily for 4 weeks. The full ingredient list for these products is provided in Table S1.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of the Global Medical Research Center (IRB number GMRC-18614-E1R). Before the trial, the composition, side effects, and benefits of the product were explained to all participants, and informed consent was obtained from the participants.

2.2 | Evaluation of clinical efficacy

The clinical efficacy of Ato Repair Solutions after 4 weeks of using the product was evaluated at baseline, week 2, and week 4. Cutometer Dual MPA580 (Courage Khazaka electronic GmbH, Köln, Germany), Ultrascan UC22 (Courage + Khazaka electronic GmbH), and Antera 3D CS (Miravex, Dublin, Ireland), Tewameter TM300 (Courage Khazaka electronic, GmbH) were used to measure skin density, skin elasticity, changes in the depth of periorbital wrinkles, and TEWL, respectively. Adverse events were also recorded, and participants were asked to rate their satisfaction with the efficacy of the products using a questionnaire at the end of the trial of 4 weeks (Table S2). The questionnaire evaluation was conducted on a five-point scale (very satisfied = 5, satisfied = 4, slightly satisfied = 3, no change = 2, not satisfied = 1), and the scores of 3 or more were considered positive responses and presented as a result.

2.3 | ZAG peptide and cell culture

The ZAG peptide (Sequence: EDWKQDSQLQKAREDIFMETLKDIVEYYND) was purchased from AbClon Inc. (Seoul, Korea). A human epidermal keratinocyte (KC; Primary Epidermal Keratinocyte; Normal; Human; Adult, PCS-200-011) cell and human dermal fibroblast (HDF) cells were purchased from ATCC (Manassas, VA, USA). The KC and HFD cells were cultured in KBM™ Gold Basal Medium (Lonza, Basel, CHE) containing KGM™ Gold SingleQuots™ supplements (Bullet Kit; Lonza) and Dulbecco's modified Eagle's Medium (Lonza, Walkersville, MD, USA) containing 10% (*v/v*) fetal bovine serum (Gibco, Grand island, NY, USA) and 1% Penicillin-Streptavidin (Gibo), respectively. All cells were cultured in a 5% CO_2 incubator at 37°C with a humidified atmosphere.

2.4 | Cell cytotoxicity measurement

Cell cytotoxicity was measured using the Cell Counting Kit-8 (CCK-8; Dojindo, Mashiki, Japan). KC cells (5×10^4 cells/well) were seeded in a 96-well cell culture plate and incubated for 24 h. ZAG peptide was added at five different concentrations (0.25, 0.5, 1.0, 2.0, and 4.0 μg/mL), or cells were irradiated with ultraviolet B (UVB) using a UVB lamp (BLX26, BIO-LINK-Crosslinker, FRA) for the induction of photo-senescence. Absorbance at 450 nm was measured using an ELISA microplate reader (VersaMax; Molecular Devices, California, CA, USA).

TABLE 1 Primer sequences used in the present study.

Gene	Forward primer	Reverse primer
p16	CTCGTGCTGATGCTACTGAGGA	GGTCGGCGCAGTTGGGCTCC
p21	AGGTGGACCTGGAGACTCTCAG	TCCTCTTGGAGAAGATCAGCCG
p53	CCTCAGCATCTTATCCGAGTGG	TGGATGGTGGTACAGTCAGAGC
MMP2	AGCGAGTGGATGCCGCCTTTAA	CATTCCAGGCATCTGCGATGAG
MMP3	CACTCACAGACCTGACTCGGTT	AAGCAGGATCACAGTTGGCTGG
MMP9	CCCTGGAGACCTGAGAACCA	CCCGAGTGTAACCATAGCGG
TIMP1	TTCCACAGGTCCCACAAC	CGTCCACAAGCAATGAGT
FLG	GCTGAAGGAACTTCTGGAAAAGG	GTTGTGGTCTATATCCAAGTGATC
IVN	GGTCCAAGACATTCAACCAGCC	TCTGGACACTGCGGGTGGTTAT

Abbreviations: FLG, filaggrin; IVN, involucrin.

2.5 | Senescence-associated beta-galactosidase (SA-β-Gal) analysis

The SA-β-Gal activity was measured using the Senescence Cells Histochemical Staining Kit (CS0030, Sigma–Aldrich) according to the manufacturer's instructions. KC cells (5×10^4 cells/well) were seeded in 6-well plates and treated with 100 mJ/cm^2 UV irradiation and 2.0 μg/mL ZAG peptide. The plates were washed with phosphate-buffered saline (PBS) and fixed with a fixation buffer for 7 min at room temperature (20–25°C). Post-fixation, the cells were washed three times with PBS and added to the staining mixture. The stained cells were incubated overnight at 37°C without CO_2 and then observed using a microscope (BX43, Olympus, Tokyo, Japan) and analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.6 | Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA were extracted from KC cells cultured for 24 h (Control; non-treatment, UV; 100 mJ/cm^2 UVB, UV+ZAG; 100 mJ/cm^2 UVB + 2.0 μg/mL ZAG peptide) and HDF cells cultured for 3 days (Control; non-treatment, UV; 10 J/m^2 UVA, UV+ZAG; 10 J/m^2 UVA + 2.0 μg/mL ZAG peptide) using TRIzol reagent (Invitrogen, Waltham, MA, USA) and processed for RNA quality control using a NanoDrop spectrophotometer (ThermoFisher Scientific, Carlsbad, CA, USA). cDNA was synthesized using the RNA to cDNA EcoDry Premix Kit (Takara Sake, Berkley, CA, USA) following the manufacturer's instructions. qRT-PCR was performed using specific primer pairs (Table 1) and SYBR Green Master MIX (Promega Co., Madison, WI, USA). *GAPDH* was used as a housekeeping gene to normalize mRNA expression levels and relative mRNA expression levels were estimated using the 2-ΔΔCt method.

2.7 | Immunofluorescence staining

KC cells (2.5×10^4 cells/well) were seeded in 4-well chamber slides and incubated for 24 h before treatment with UVB (10 J/cm^2) or UVB (10 J/cm^2) + ZAG peptide (2.0 μg/mL). Cells were then fixed with 4% (w/v) paraformaldehyde (Cell Signaling Technology, Danvers, MA, USA) for 15 min at room temperature, followed by three washes with PBS. The fixed cells were incubated overnight with anti-loricrin (Abcam Cambridge, UK) or anti-filaggrin (Abcam) antibodies at 4°C. Thereafter, the slides were washed three times and incubated with FITC-labeled secondary fluorescent antibodies (Goat pAb to Rb IgG-FITC; Abcam) for 2 h at room temperature. Finally, the slides were fixed with VECTASHIELD mounting medium with 4′,6-diamidino-2-phenylindole (Vector Laboratories Inc., Burlingame, CA, USA). Fluorescence images were obtained using a laser-scanning microscope (LSM 700, Carl Zeiss, Jena, Germany) and analyzed using ImageJ software.

2.8 | Data analysis

All data are expressed as means ± standard deviations or percentages. The Kolmogorov–Smirnov test was used to verify normality. Data at baseline, week 2, and week 4 were compared using the Friedman test or Wilcoxon signed-rank or repeated-measures ANOVA, with subsequent post hoc analysis performed using Bonferroni correction. A p-value of <0.05 was considered statistically significant. All laboratory studies were conducted in triplicate ($n \geq 3$). Statistical analyses were performed using SPSS statistics version 25.0 software (IBM Corp., Armonk, NY, USA).

3 | RESULTS

3.1 | Participant characteristics

The study included 22 Asian women between the ages of 35 and 60 years who presented with conspicuous wrinkles around their eyes. The patients had a mean age of 50 ± 5.31 years. All participants successfully completed the study until the final visit, and none dropped out due to non-compliance. No adverse events were reported by patients after 4 weeks of the trial.

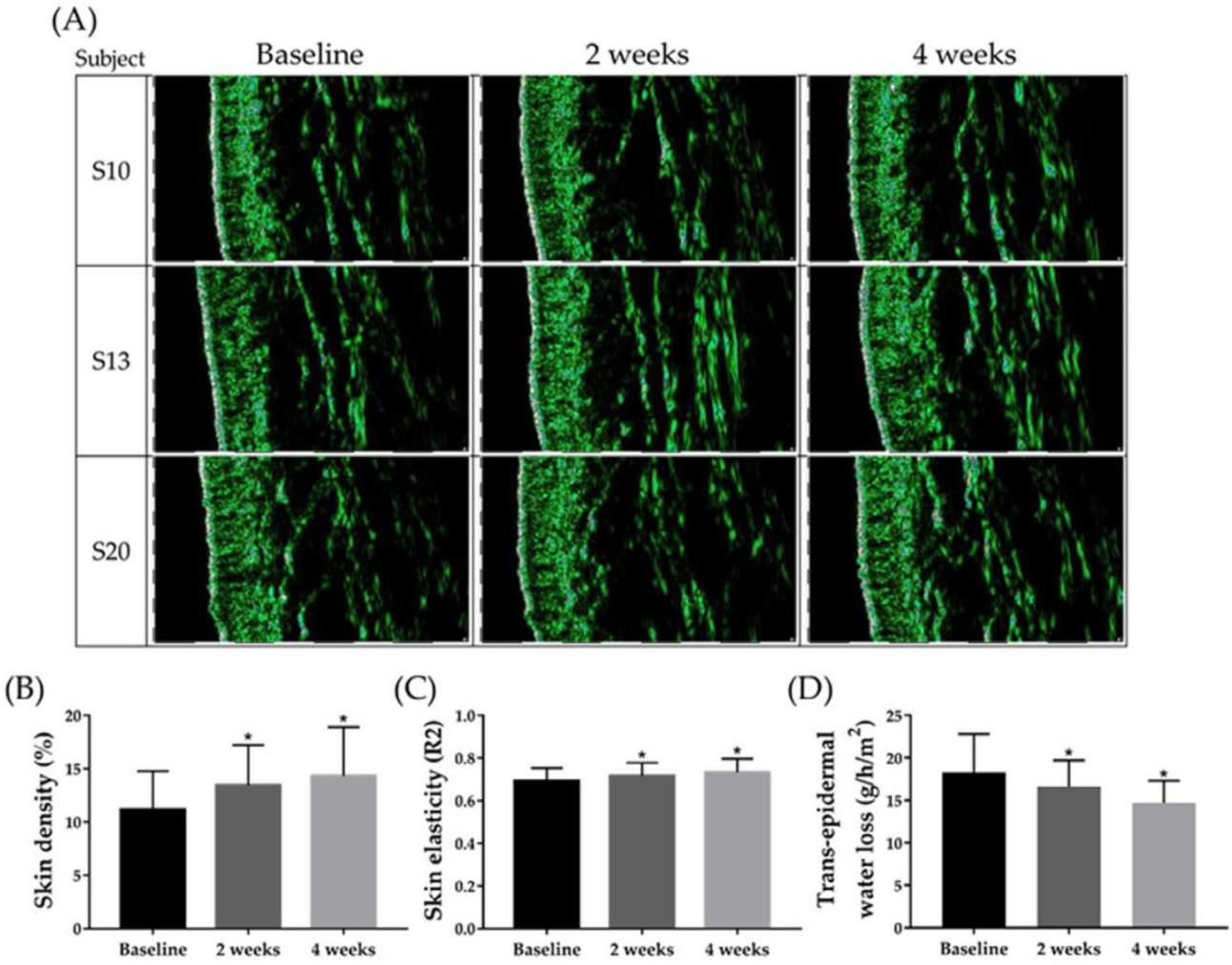

FIGURE 1 Changes in skin density and elasticity following the application of the Ato Repair Cleaner, Lotion, and Cream (Ato Repair Solutions). (A) The representative images show the increased skin density affected by Ato Repair Solutions application time-dependently. (B, C, and D) Effects of Ato Repair Solutions on (B) skin density, (C) skin elasticity and trans-epidermal water loss (D); $n = 22$; $^*p < 0.05$ estimated using repeated measures ANOVA.

3.2 | Clinical efficacy and safety of Ato repair solutions containing ZAG peptide

Measurement of the skin density and elasticity after 4 weeks of application of Ato Repair Solutions showed a significant increase in skin density (Baseline; 11.18 ± 3.60%, week 2; 13.45 ± 3.78%, week 4; 14.32 ± 4.60%, repeated measures ANOVA, $p < 0.05$, Figure 1A,B) and skin surface elasticity (R^2) (Baseline; 0.69 ± 0.06, week 2; 0.72 ± 0.06, week 4; 0.73 ± 0.06, repeated measures ANOVA, $p < 0.05$, Figure 1C). In Figure 1D, the TEWL measurement result was shown. According to the application weeks, the TEWL was decreased than the baseline (Baseline; 18.281 ± 4.517, week 2; 16.622 ± 3.095, week 4; 14.700 ± 2.592, repeated measures ANOVA, $p < 0.05$). The depth of periorbital wrinkles (mm) was significantly decreased at weeks 2 (0.089 ± 0.030 mm) and 4 (0.082 ± 0.026 mm) compared to that at baseline (0.129 ± 0.034 mm) [$p < 0.025$ (= 5%/2); Friedman test and post hoc Wilcoxon signed rank test; Figure 2A,B]. All participants responded to the questionnaire on subject satisfaction score (Figure 2C). The questionnaire assessment of the satisfaction scores of the participants showed that 86%, 86%, and 91% of participants rated the improvements in skin density, skin elasticity, and periorbital wrinkles, respectively, as satisfactory, with a score of ≥ 3. No adverse events were reported by any of the patients during the study, and no patients dropped out due to adverse events. These results suggest that the treatment with Ato Repair Solutions containing ZAG peptide was safe.

3.3 | Evaluation of the effect of ZAG peptide on cell cytotoxicity and cellular aging

As shown in Figure 3, the viability of KC cells treated with 2.0 μg/mL ZAG peptide and 100 mJ/cm^2 UVB irradiation was 90.26 ± 4.76% and 79.16 ± 11.67%, respectively (Figure 3A,B). Therefore, we used these treatment conditions for subsequent experiments).

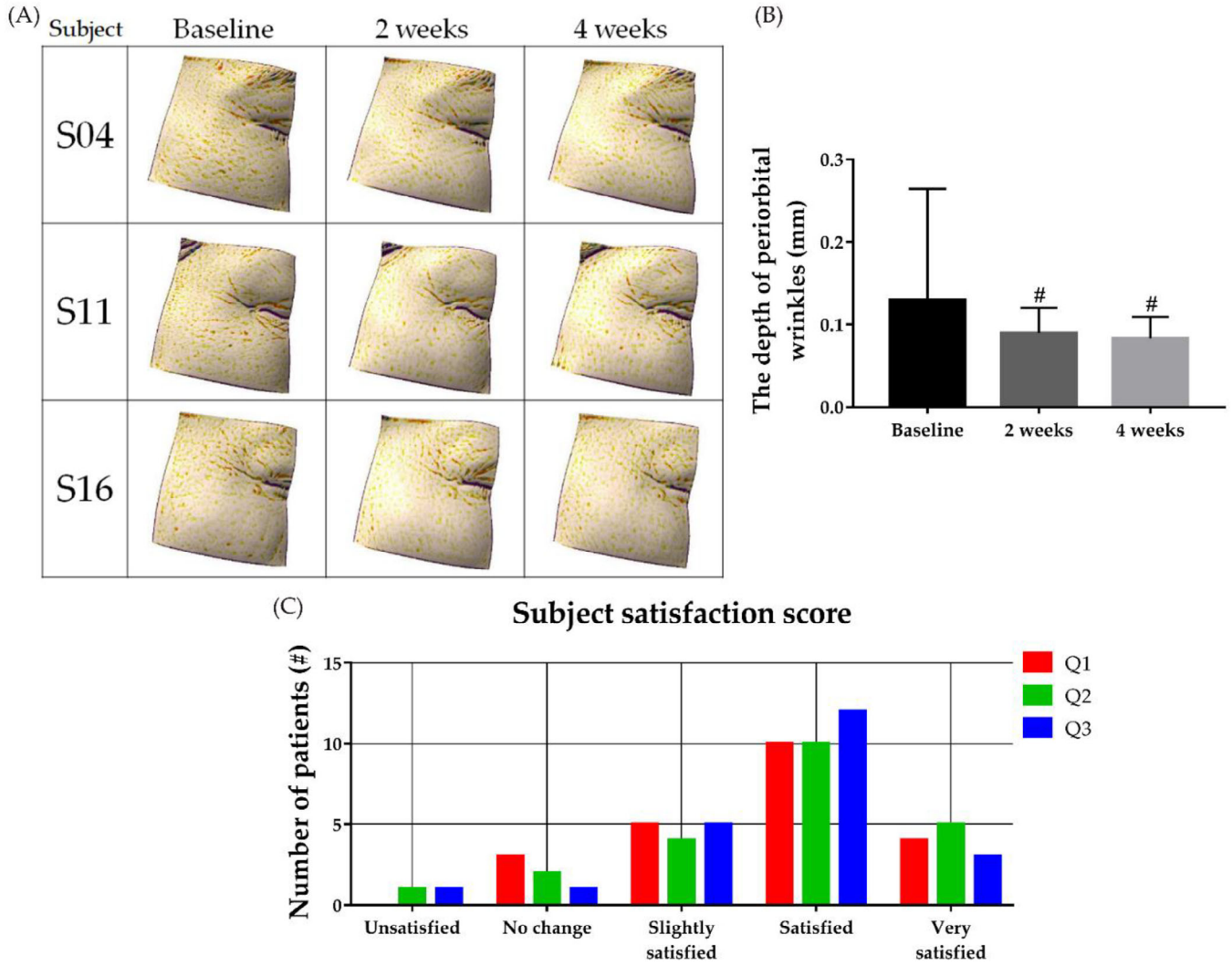

FIGURE 2 Effects of the application of Ato Repair Solutions on the depth of the periorbital wrinkles. (A) Representative images from three individual patients showing the depth of the periorbital wrinkles at baseline and weeks 2 and 4; (B) quantification of the depth of the periorbital wrinkles; $n = 22$; $^{\#}p < 0.025$ (= 5%/2) obtained using Freidman test and post hoc Wilcoxon signed-rank test. (C) The satisfaction score of the participants who responded to the questionnaires: Q1, improvement of the skin density; Q2, improvement of the skin elasticity; Q3, improvement of the depth of the periorbital wrinkles.

The representative images of SA-β-gal staining in KC cells are shown in Figure 4A. The ratio of SA-β-gal stained cells was significantly higher in the UVB-treated group (36.79 $\pm$ 1.99%) than that in the control group (16.75 $\pm$ 1.62%) ($p < 0.0005$; Figure 4B). On the contrary, ZAG peptide treatment alleviated the UVB-induced increased senescence; the ratio of SA-β-gal staining cells was significantly reduced to 23.68 $\pm$ 2.70%.

Next, we evaluated the effects of ZAG peptide on the expression of genes related to cellular senescence. qRT-PCR analysis revealed that the mRNA expression of *p16*, *p21*, and *p53* was significantly higher in the UVB irradiation group than those in the control group (Figure 4C). However, ZAG peptide treatment of the UVB-irradiated KC cells significantly reduced the expression levels of these mRNAs. In contrast, UVB irradiation increased the expression of matrix metallopeptidase (*MMP*) *2* and *MMP9*, which was significantly reduced by treatment with ZAG peptide (Figure 4C). The expression of tissue inhibitor of metalloproteinases1 (*TIMP1*) was also reduced in the UVB irradiation group compared with that in the control group, which was improved by treatment with ZAG peptide (Figure 4C). These results confirm the possible anti-aging effect of the ZAG peptide treatment on KC cells aged by UVB irradiation.

3.4 | Evaluation of skin barrier improvement effect of ZAG peptide in vitro

The potential of ZAG peptide to improve skin barrier was evaluated using immunofluorescence staining and qRT-PCR. The results demonstrated the reduced staining intensity of *FLG* in the UVB-irradiation group compared with that in the control group (Figure 5A,B). In

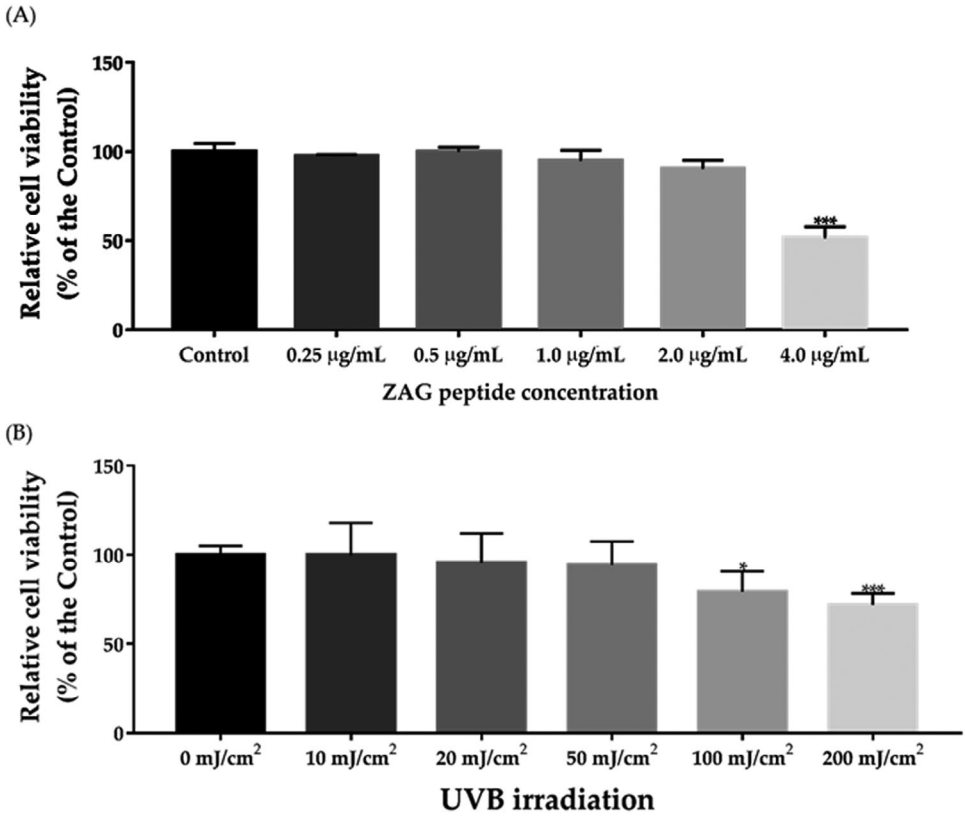

FIGURE 3 Relative cell viability after treatment of keratinocyte (KC) cells with ZAG peptide or UVB irradiation. (A, B) Viability of the KC cells treated with the indicated concentrations of (A) ZAG peptide and (B) and doses of UVB measured using a cell counting kit. $^{*}p < 0.05$, $^{***}p < 0.005$ vs. Control estimated using independent samples *t*-test.

contrast, the staining intensity of *FLG* was significantly increased in the ZAG peptide treatment group compared with that in the UVB-irradiation group (Figure 5A,B). Similar results were also observed for the mRNA expression of *FLG*. Furthermore, treatment with ZAG peptide improved the UVB-induced reduction in staining intensity (Figure 5A,B) and mRNA expression of involucrin (*IVN*; Figure 5C). These results suggest that treatment with ZAG peptide improves the skin barrier function in vitro.

4 | DISCUSSION

Skin aging is a complex process influenced by both intrinsic and extrinsic factors. As we age, our skin gradually loses collagen and elastin, essential for maintaining skin density,[10] resulting in thinner and less elastic skin, leading to wrinkles, sagging, and other signs of aging. Skin density is a determining factor in maintaining the ability of the skin to retain moisture and heal, regenerate, and protect the body.[11] Hence, poor skin density can lead to dry skin, accelerating the signs of aging. Our study investigated the effects of the ZAG peptide on skin aging and barrier function using in vitro and clinical assessments. The clinical evaluation of Ato Repair Solutions containing ZAG peptide as the main ingredient in patients with noticeable periorbital wrinkles revealed its potential to improve skin density and elasticity and reduce the depth of periorbital wrinkles. Furthermore, using the KC cells in in vitro experiments, our results showed that ZAG peptides are safe to use and exert anti-aging and skin barrier improvement effects. These results suggest that Ato Repair Solutions are effective anti-aging agents for treating noticeable periorbital wrinkles.

SA-β-gal is associated with the aging process of animal cells and is considered one of the primary causes of skin aging.[12] Its role in the breakdown of collagen and other molecules that make up the extracellular environment of the skin contributes to skin aging.[13] In this study, we showed that treatment with ZAG peptide significantly reduced the ratio of SA-β-gal-stained cells in UVB-irradiated KC cells. These results suggest the potential of the ZAG peptide to reduce the UVB irradiation-induced aging in KC cells by inhibiting the activity of SA-β-gal.

p16, *p21*, and *p53* are closely associated with senescence and skin aging. The increased expression of *p16* is associated with decreased collagen production, which leads to wrinkle formation in aged skin.[14] The expression of *p21* is associated with accumulating damaged proteins and forming senescent cells, leading to age-associated wrinkles and increased skin elasticity.[15,16] The association of the expression of *p53*, which is responsible for regulating cell cycle progression and differentiation, with cellular senescence and the development of wrinkles is evident in previous studies.[17–19] Our results showed that the expression of these genes was significantly reduced in the

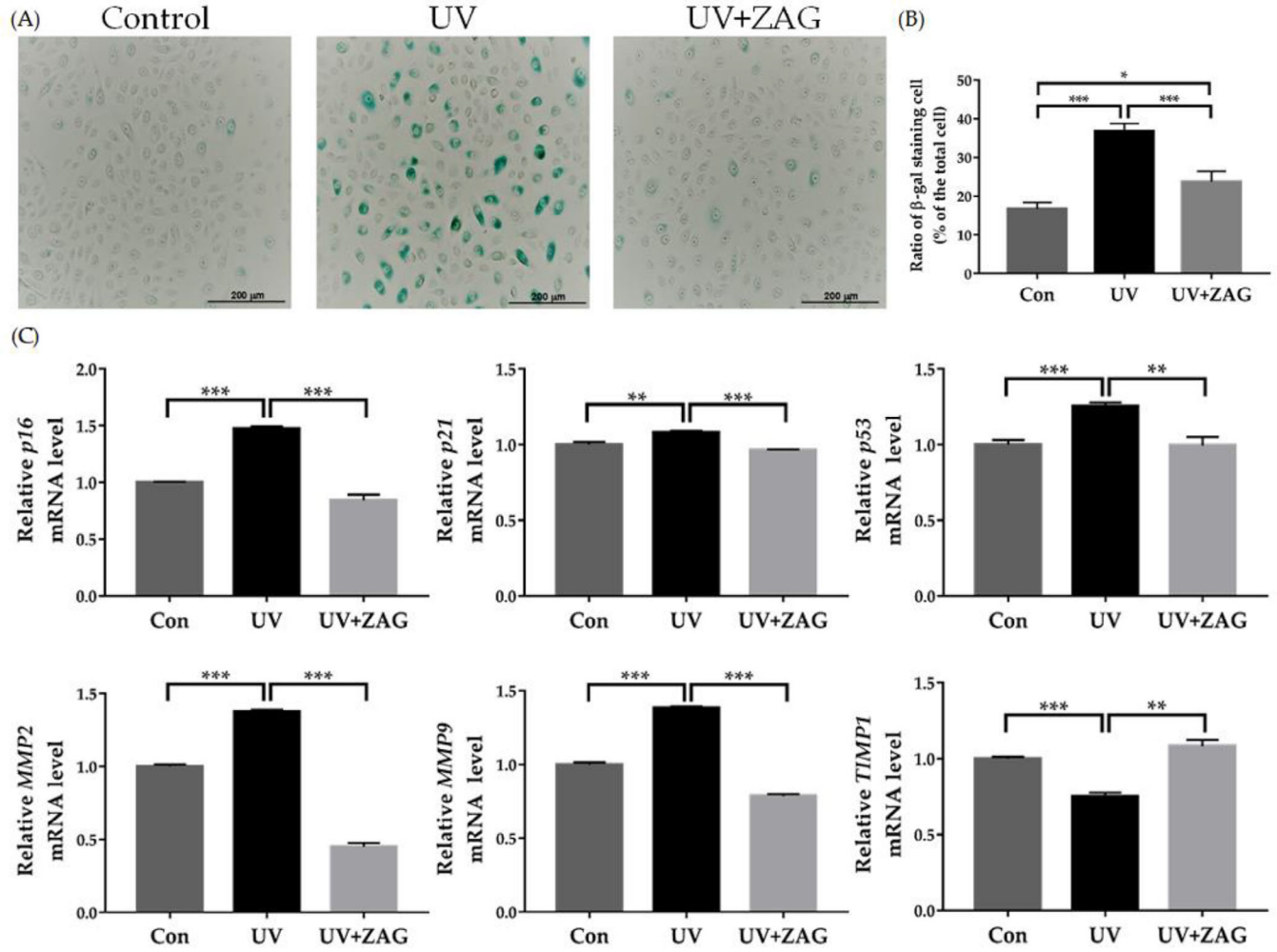

FIGURE 4 ZAG peptide alleviates the UVB-induced senescence and alterations in the relative expression of mRNAs associated with senescence (A) Representative images of senescence-associated beta-galactosidase (SA-β-gal) staining; (B) quantification of the SA-β-gal stained cells (ratio of SA-β-gal stained cells/total cells). (C) Effects of ZAG peptide on relative expression of the mRNAs of senescence-related factors; *MMP2*, Matrix Metallopeptidase 2; *MMP9*, Matrix Metallopeptidase 9; *TIMP1*, Tissue inhibitor matrix metalloproteinase 1; Con, Control group; UV, 100 mJ/cm^2 UVB treatment group; UV+ZAG, 100 mJ/cm^2 UVB treatment plus 2.0 μg/mL ZAG peptide; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ compared with the Control or UV groups estimated using independent samples *t*-test.

ZAG peptide-treated group compared with that in the UVB-irradiated group, suggesting that the attenuation of senescence in KC cells by ZAG peptide is mediated via inhibition of the expression of *p16*, *p21*, and *p53*, the aging-related genes.

MMP2, *MMP9*, and *TIMP1* are also associated with collagen degradation that contributes to skin aging. MMP2 and MMP9 proteins degrade the interface between proteins, collagen, and elastin, which are responsible for the structural strength of the skin.[20,21] TIMP1 counteracts MMPs and preserves the structure of the skin.[22] Here, we demonstrated that the ZAG peptide reduced the expression of *MMP2* and *MMP9* and induced the expression of *TIMP1* in KC cells subjected to UVB irradiation, suggesting that the anti-aging effects of the ZAG peptides were modulated via the regulation of these genes. Moreover, skin aging is caused in whole skin containing KC and fibroblast cells, the MMP3, MMP9, and p53 gene expression of UVA irradiation plus ZAG peptide treatment group was significantly reduced than the UVA irradiation group on HDF cells, which shows that the ZAG peptide treatment has the anti-aging effects on not only KC cell but also fibroblast (Figure S1).

The skin barrier protects the skin from environmental damage, like UV radiation and harsh chemicals, and prevents water loss, immune reaction, and inflammation. FLG is an essential protein for forming the skin barrier and preventing water loss, while IVN is involved in producing epidermal lipids, which help keep the skin hydrated.[23,24] Our results showed that the ZAG peptide increased the staining intensity of FLG and IVN in the UVB-irradiated KC cells and increased the mRNA expression of *FLG* and *IVN*. These findings suggest that the ZAG peptide has the potential to improve skin barrier function, reduce wrinkles and TEWL.

Even though this study provides promising results on the potential anti-aging effects of the ZAG peptide, there are several limitations to be considered. Firstly, this study was conducted using in vitro

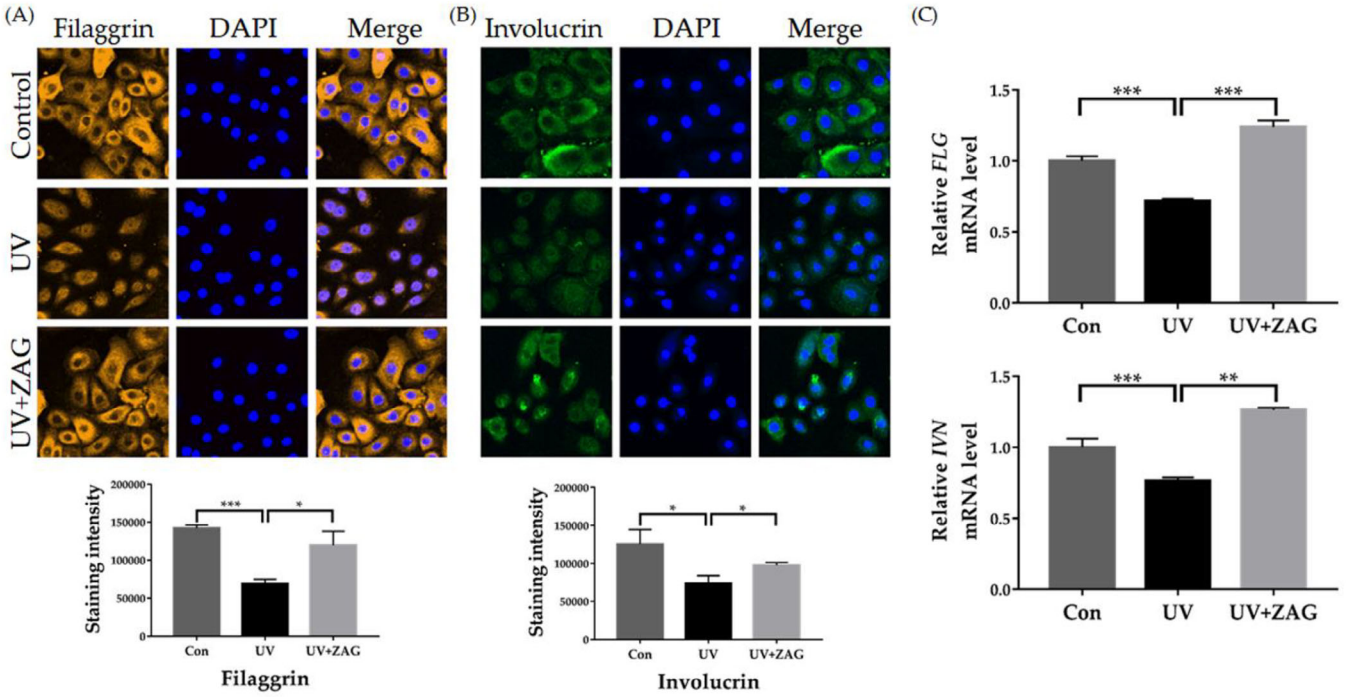

FIGURE 5 ZAG peptide treatment improves the skin barrier function. (A, B) The representative images of immunofluorescence staining using (A) ant-filaggrin and (B) anti-involucrin antibodies. The bar graphs below respective images show the quantification results of staining intensity, estimated using Image J. (C) Effects of ZAG peptide on the expression of *FLG* and *IVN* mRNAs in keratinocyte cells. *FLG*, filaggrin; *IVN*, involucrin; Con, Control group; UV; 100 mJ/cm^2 UVB treatment group; UV+ZAG, 100 mJ/cm^2 UVB treatment plus 2.0 μg/mL ZAG peptide; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, independent samples *t*-test compared with the Control group or UV group.

experiments and a clinical trial with a relatively small sample size. Therefore, larger clinical trials with more diverse populations are needed to further validate the efficacy of ZAG peptides against skin aging. Additionally, the detailed mechanism underlying the anti-aging effects of the ZAG peptide remains unclear, and further research is required to fully understand the molecular mechanisms involved. Finally, this study focused on the effects of the ZAG peptide on the skin barrier, collagen degradation, and senescence. However, the effects of the ZAG peptide on other aspects of skin aging, such as pigmentation and inflammation, were not investigated.

In conclusion, our study provides evidence that the ZAG peptide may be a promising therapeutic agent for skin aging. The application of Ato Repair Solutions containing ZAG peptide improved skin density, elasticity, and wrinkles in a clinical setting. Our in vitro experiments demonstrated that ZAG peptide exerts potential anti-aging effects on KC cells by reducing the activity of SA-β-gal and attenuating the induction of senescence by inhibiting the expression of *p16*, *p21*, and *p53*. Additionally, the ZAG peptide reduced the expression of *MMP2* and *MMP9* and induced that of *TIMP1*, which may contribute to its anti-aging effects. Finally, the ZAG peptide increased the expression of *FLG* and *IVN*, important components of the skin barrier function. Taken together, according to the skin barrier was improved with increased FLG and IVN expression, the skin density and elasticity were increased and the TEWL and wrinkles were reduced. Additionally, in the results of gene expression levels, aging, and wrinkle-related genes were improved. These results are evidence of the anti-aging mechanism of the ZAG peptide effects we want to suggest. Nevertheless, validation of our findings to explore the therapeutic benefits of ZAG peptides and understanding the exact mechanism underlying its anti-aging effects warrants further research.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Tobin DJ. Introduction to skin aging. *J. Tissue Viability*. 201726:37-46.
2. Khavkin J, Ellis DA. Aging skin: histology, physiology, and pathology. *Facial Plast Surg Clin North Am*. 201119:229-234.

3. Coleman SR, Grover R. The anatomy of the aging face: volume loss and changes in 3-dimensional topography. *Aesthet Surg J*. 200626: S4-S9.
4. Farage M, Miller K, Elsner P, et al. Intrinsic and extrinsic factors in skin ageing: a review. *Int. J. Cosmet. Sci*. 200830:87-95.
5. Rawlings A, Harding C. Moisturization and skin barrier function. *Dermatol Ther*. 200417:43-48.
6. Panich U, Sittithumcharee G, Rathviboon N, et al. Ultraviolet radiation-induced skin aging: the role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. *Stem Cells Int*. 20162016:1.
7. Hassan MI, Waheed A, Yadav S, et al. Zinc α2-glycoprotein: a multidisciplinary protein. *Mol. Cancer Res*. 20086:892-906.
8. Brysk MM, Lei G, Adler-Storthz K, et al. Zinc-α2-glycoprotein expression as a marker of differentiation in human oral tumors. *Cancer Lett*. 1999137:117-120.
9. Noh JY, Shin JU, Kim JH, et al. ZAG regulates the skin barrier and immunity in atopic dermatitis. *J. Invest. Dermatol*. 2019139:1648-1657.e7.e1647.
10. Daly CH, Odland GF. Age-related changes in the mechanical properties of human skin. *J. Invest. Dermatol*. 197973:84-87.
11. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. *Photochem. Photobiol*. 200884:539-549.
12. Choi J, Shendrik I, Peacocke M, et al. Expression of senescence-associated beta-galactosidase in enlarged prostates from men with benign prostatic hyperplasia. *Urology*. 200056:160-166.
13. Ryu HS, Joo YH, Kim SO, et al. Influence of age and regional differences on skin elasticity as measured by the Cutometer®. *Skin Res Technol*. 200814:354-358.
14. Hall BM, Balan V, Gleiberman AS, et al. Aging of mice is associated with p16 (Ink4a)-and β-galactosidase-positive macrophage accumulation that can be induced in young mice by senescent cells. *Aging (Albany NY)*. 20168:1294.
15. Chang B-D, Watanabe K, Broude EV, et al. Effects of p21Waf1/Cip1/Sdi1 on cellular gene expression: implications for carcinogenesis, senescence, and age-related diseases. *Proc. Natl. Acad. Sci. U.S.A*. 200097:4291-4296.
16. Ahn JJ, Yong HJ. Antioxidant and inhibition of senescence effects of embelin. *Biomed. dermatol*. 20182:1-8.
17. Baumann L. Skin ageing and its treatment. *J. Pathol*. 2007211:241-251.
18. Chandler H, Peters G. Stressing the cell cycle in senescence and aging. *Curr. Opin. Cell Biol*. 201325:765-771.
19. Wu H, Wade M, Krall L, et al. Targeted in vivo expression of the cyclin-dependent kinase inhibitor p21 halts hepatocyte cell-cycle progression, postnatal liver development and regeneration. *Genes Dev*. 199610:245-260.
20. Pittayapruek P, Meephansan J, Prapapan O, et al. Role of matrix metalloproteinases in photoaging and photocarcinogenesis. *Int. J. Mol. Sci*. 201617:868.
21. Kähäri VM, Saarialho-Kere U. Matrix metalloproteinases in skin. *Exp. Dermatol*. 19976:199-213.
22. Herouy Y, Mellios P, Bandemir E, et al. Inflammation in stasis dermatitis upregulates MMP-1, MMP-2 and MMP-13 expression. *J. Dermatol. Sci*. 200125:198-205.
23. Sandilands A, Sutherland C, Irvine AD, et al. Filaggrin in the frontline: role in skin barrier function and disease. *J. Cell Sci*. 2009122:1285-1294.
24. Ekanayake-Mudiyanselage S, Jensen J-M, Proksch E, et al. Expression of epidermal keratins and the cornified envelope protein involucrin is influenced by permeability barrier disruption. *J. Invest. Dermatol*. 1998111:517-523.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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