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"Zinc Glycinate boosts holistic beauty of scalp & hair"

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1. Introduction

Zinc is an essential micronutrient for the maintenance of human biological functions. Furthermore, the skin is recognized as one of the most zinc-rich tissues in the body, followed by the bone and muscle. In particular, epidermal cells possess metallothioneins that serve as zinc-binding proteins, resulting in a high concentration of zinc in the epidermis. Zinc plays a pivotal role in stabilizing and regulating the functions of various biomolecules, including genes, proteins, and enzymes, and is crucial for maintaining epidermal homeostasis [1]. Zinc deficiency can lead to a range of dermatological issues. Both dietary and genetic zinc deficiencies have been implicated in the development of conditions such as alopecia and vesicular or pustular dermatitis. Prior research has demonstrated that knockdown of Zip2, a zinc transporter in epidermal cells, results in a reduction of involucrin expression, further emphasizing the importance of zinc in skin function [2]. Consequently, zinc is regarded as a crucial metal in skin care.

In a previous study, we screened various zinc compounds and reported that Zinc Glycinate (ZnGly), a water-soluble amino acid complex, has significant potential for facial care. This compound enhances the resilience of epidermal cells, thereby preventing wrinkle formation and pigmentation induced by ultraviolet (UV) radiation [3,4]. In addition to our, other studies have focused on the anti-acne effects of ZnGly [5]. However, few studies have explored the application of ZnGly in scalp and hair care, with pyrithione zinc being the most widely studied compound in this regard. In the dandruff treatment domain, shampoos containing pyrithione zinc dominate the market [6].

To date, there have been no reports of zinc compounds that simultaneously approach scalp care and hair care. In the present study, we investigated the effects of ZnGly on dandruff in a clinical trial involving participants with dandruff. Subsequently, we evaluated the protective effects of ZnGly against hair damage, such as loss of elasticity and strength induced by UV radiation. As the objective of this study was to simultaneously target both the scalp and hair, the clinical trial involved the use of a lotion formulation containing ZnGly. In contrast, when human hair is exposed to sunlight, various hair damage such as deterioration of the cuticle,

loss of tensile strength, changes in hair color and loss of moisture is caused [7–9]. Furthermore, UV-induced hair damage occurs through the carbonylation of hair proteins and an increase in porosity. In particular, carbonylation of hair proteins is considered the first stage of hair damage; therefore, it is important to prevent carbonylation [10,11]. We investigated a novel method for detecting carbonylated proteins in hair by combining hair protein extraction with an enzyme-linked immunosorbent assay (ELISA) [12]. In this study, we evaluated the efficacy of ZnGly in the suppression of UV damage by comparing it to other zinc compounds. Also, we evaluated the localization of zinc on the hair by SEM-EDX (scanning electron microscopy with energy dispersive X-ray spectroscopy) and using fluorogenic Zn^{2+} reporter.

2. Materials and Methods

2.1. Cell Culture

Normal human epidermal keratinocytes (NHEKs) were purchased from Kurabo Industries NHEKs were grown routinely in the low-Ca^{2+} medium HuMedia-KG2 supplemented with growth factor cocktail (Kurabo Industries) for propagation at 37°C in a humidified atmosphere containing 5% CO_2.

2.2. TEER measurement

NHEKs were seeded onto cell culture inserts (Corning) for 24 hours and grown to confluence. After 72 hours of incubation with the test sample, the transepithelial electrical resistance (TEER) was measured using a Millicell-ERS Voltohmmeter (Millipore).

2.3. Clinical trial test to evaluate the anti-dandruff effects of ZnGly

The anti-dandruff effects of ZnGly were tested in a clinical trial. The participants included 24 healthy Chinese individuals with sensitive scalps and mild to moderate dandruff level (scalp itching, tingling, and redness) scores on any indicator by expert assessment, and were aged 24–60 years. Twenty-four participants were divided into two groups: one using a placebo lotion and the other using 1% ZnGly lotion. They used the product twice daily, in the morning and evening. The evaluation items were the sensitive scalp score and number of dandruffs. Sensitive scalp score was scored by the assessment experts in the following ranges: Calculated by summing each score with reference to a previous study (Total score: 16; dandruff 0–10, redness 0–3, itchiness 0–3) [13]. Dandruff was collected on a black cloth in a Petri dish by combing the participant's hair for 10 minutes, measured using DandruffMeter DA20 (Courage + Khazaki electronic GmbH), and counted as dandruff. This clinical trial was performed in accordance with the principles of the Declaration of Helsinki.

2.4. Preparation of UV-irradiated hair samples

Human black hair (30 cm, 10 g, BS-B3A, Beaulax) was immersed in an aqueous solution of ZnGly (0.5 %), zinc aspartate, zinc pyrrolidone carboxylic acid, or zinc citrate at the same zinc molar concentration for 1 hour at room temperature. Subsequently, the hair was exposed to UV light with a wavelength of 295 nm to 315 nm and an intensity of 360 μW/cm² for 10 hours (13 J/cm²) using a UVB Broadband TL lamp (Philips). An exposure dose of 13 J/cm^2 is equivalent to the approximate exposure to sunlight around noon in midsummer for 1 hour (20 J/cm^2).

2.5. Inhibitory effect of UV irradiation on the production of carbonyl hair proteins

UV-irradiated hair was immersed in a solution containing urea/thiourea/dithiothreitol at a concentration of 50 mg/mL and incubated at 50°C for 24 hours. The solution was centrifuged (15,000 rpm, 10 minutes, 20°C), and the supernatant was used as the hair protein solution.

The protein content of the hair solution was measured using the Bradford method (Dojindo Laboratories). The amount of carbonylated protein in the hair protein solution was measured using an OxiSelect Protein Carbonyl ELISA Kit (STA-310, Cell Biolabs).

2.6. Zinc adsorption evaluation on hair surface by SEM-EDX

Zinc adsorption by hair treated with a solution of zinc compounds (without UV irradiation) was evaluated using SEM-EDX. Elemental analysis of the hair surface was performed using a tabletop SEM (Miniscope TM4000, Hitachi High-tech) and a Bruker Quantax (Bruker). A certain area of the hair surface was scanned at 1,800 x magnification, and a spectral image was obtained using spectroscopy and characteristic X-ray detection. The amount of zinc was calculated from the peak area value as the zinc ratio, assuming that the main elements in hair are carbon, nitrogen, oxygen, sulfur, and zinc.

2.7. Zinc adsorption evaluation on hair surface by fluorogenic Zn^{2+} reporter

The localization of zinc on the hair surface was observed using Zinpyr-1 (TRC-Z440000, TRC). Zinpyr-1 is a cell-permeable fluorescein-based zinc detection probe, and is used to detect the localization of zinc in biological tissues. Hair treated with a zinc compound solution (without UV irradiation) was immersed in 5 µmol/L Zinpyr-1 in PBS-T and incubated at room temperature for 3 minutes. Hair samples were washed twice with PBS-T and dried at room temperature. The hair surface was observed and thin sections were cut with a microtome and observed under a confocal laser microscope (LSM 800, Carl Zeiss) at a magnification of 100x and a laser wavelength of 488 nm.

2.8. Statistical analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed by Student's t-test, paired t-test, and Dunnett's test using SPSS software. The Mann-Whitney U test and Analysis of covariance (ANCOVA) were used for data measured on an ordinal scale of measurement (sensitive scalp score) and ratio scales of measurement (dandruff count), respectively. Statistical significance was set at $p<0.050$.

3. Results

3.1. Effects of ZnGly on TEER in NHEKs

To investigate the effects of ZnGly on epidermal barrier function, we measured the TEER in NHEKs. The TEER values of NHEKs treated with ZnGly were significantly higher than those of the negative control NHEKs. The results are shown in Figure 1. As ZnGly increased the TEER of NHEKs, we hypothesized that it might promote epidermal barrier function.

3.2. Anti-Dandruff Effects of ZnGly

In total, 24 participants completed the study. The values of the sensitive scalp symptoms measured using a score of 16 points during the test period are shown in Figure 2. Scalp sensitive symptoms decreased from 6.3 ± 1.8 to 2.7 ± 1.0 in the ZnGly group. When comparing the delta value (Δ4w-0w), the ZnGly group had significantly lower value scalp roughness symptoms than the placebo group ($p=0.008$). The amount of dandruff measured during the test

period is shown in Figure 3. The amounts decreased from 183.2 ± 25.1 to 56.5 ± 45.5 in the ZnGly group. In the comparison of the delta value (Δ4w-0w), the ZnGly group had significantly lower amount of dandruff than the placebo group ($p<0.001$). These results indicate that ZnGly is expected to improve sensitive scalp conditions.

3.3. Inhibitory effect of UV irradiation on the production of carbonyl hair proteins

The amount of carbonylated protein in the protein solution extracted from UV-irradiated hair is shown in Table 1. The amount of carbonylated proteins significantly increased when unirradiated hair was subjected to UV irradiation. Furthermore, carbonylated proteins were observed in hair immersed in an aqueous ZnGly solution and did not increase to the same level as in untreated hair. The amount of carbonylated protein in hair pretreated with zinc compounds other than glycinate was at the same level as that in irradiated hair and blank hair (water), and no oxidative damage suppressive effect similar to that of ZnGly was observed.

Table 1. Carbonylation level in hair protein treated with each aqueous Zn compound after UV irradiation

Treatment	UV	Carbonylated protein (mmol/μg)		p1 (vs. Blank)
		Mean	S.D.	
-	-	1.179	0.124	-
-	+	1.560	0.127	-
Blank (water)	+	1.543	0.227	-
ZnGly	+	1.040	0.167	0.005
ZnAsp	+	1.593	0.403	0.990
ZnPCA	+	1.576	0.385	0.998
ZnCa	+	1.675	0.293	0.774

3.4. Zinc adsorption evaluation on hair surface by SEM-EDX

The zinc ratio on the hair surface, calculated using SEM-EDX, is shown in Table 2. A higher relative zinc concentration was detected in hair treated with ZnGly than that in untreated hair or blank hair(water). In addition, the amount of zinc present on the surface of hair treated with zinc compounds without glycine was lower than that on hair treated with ZnGly.

Table 2. Zn ratio in hair cuticles after treatment with various aqueous Zn compounds

Treatment	Zn ratio (%)		p1 (vs. Blank)	p2 (vs. ZnGly)
	Mean	S.D.		
-	0.023	0.006	1.000	<0.001
Blank (water)	0.037	0.031	-	<0.001
ZnGly	0.844	0.205	<0.001	-
ZnAsp	0.258	0.059	0.008	<0.001
ZnPCA	0.326	0.085	0.001	<0.001
ZnCa	0.280	0.035	0.003	<0.001

3.5. Zinc adsorption evaluation on hair surface by fluorogenic Zn^{2+} reporter

Surface and internal images of hair treated with Zinpyr-1 are shown in Fig 4. On surface observation, hair ZnGly showed strong fluorescence, whereas the untreated hair or blank hair (water) images showed slight fluorescence due to autofluorescence. The results confirmed that the fluorescence of ZnGly was stronger than that of other zinc compounds without glycine. However, when observing the internal parts, no significant differences were observed between the samples.

4. Discussion

In the clinical trial, continuous application of a ZnGly lotion was found to improve sensitive scalp conditions, leading to a shift towards a healthy scalp. In particular, a significant reduction in dandruff was observed. Excessive desquamation such as dandruff is generally attributed to disturbances in epidermal turnover. In scalps with dandruff, these disruptions are thought to result from the metabolic products of fungi such as *Malassezia*, which produce free fatty acids that trigger inflammation in epidermal cells [14]. Consequently, ingredients with antifungal, anti-inflammatory, and epidermal barrier-enhancing properties are considered beneficial for improving the sensitive scalp conditions associated with dandruff. We previously demonstrated that ZnGly has anti-inflammatory effects associated with the upregulation of metallothionein expression in keratinocytes. In addition, this study confirmed the ability of ZnGly to promote epidermal barrier formation. Furthermore, although glycine is non-ionophoric and does not exhibit potent antifungal activity against compounds such as zinc pyrithione, our findings suggest that ZnGly may possess mild antifungal properties (data not shown). The combined effects of ZnGly are likely to contribute to the observed improvement in sensitive scalp conditions. Additionally, prior research has indicated that zinc levels are reduced in areas exhibiting abnormal epidermal turnover, such as in psoriasis [15]. As zinc levels in the epidermal tissue may be deficient in the dandruff-affected area, as in the case of psoriasis, it is possible that zinc supplementation has a notable positive effect on dandruff. Therefore, zinc supplementation may have beneficial effects on dandruff. Future investigations should aim to clarify the relationship between dandruff and epidermal zinc content in the scalp.

The use of a scalp lotion also results in the adhesion of the lotion to hair. Based on this observation, we hypothesized that ZnGly that adheres to hair during lotion application may exhibit hair care effects. To test this hypothesis, we evaluated the protective effects of ZnGly against UV-induced hair damage. The results revealed that ZnGly provided superior hair protection compared to other zinc compounds. This effect is likely attributable to the adsorption properties of glycine, which has an affinity for keratin. Notably, glycine alone did not mitigate UV-induced hair damage. Moreover, the adsorption of ZnGly onto hair surface can be considered as a form of storage in which the ZnGly complex is retained in the hair. Through hair care treatment, if ZnGly stored in hair provides sustained zinc supplementation to the epidermal tissue of the scalp, this may represent an innovative approach to drug delivery. Finally, we previously reported that ZnGly possesses potential anti-hyperpigmentation and anti-wrinkle effects. Consequently, skincare formulations containing ZnGly can offer a holistic solution extending from scalp and hair care to facial care.

5. Conclusion

ZnGly exhibits a dual action effect by enhancing scalp health and protecting hair from UV-induced damage. Clinical trials have demonstrated significant anti-dandruff efficacy and improved scalp comfort after ZnGly use. In vitro studies confirmed the role of ZnGly in strengthening the epidermal barrier and preventing oxidative protein damage in hair. These beneficial

effects are likely attributable to the unique zinc delivery and adsorption properties of ZnGly conferred by its glycine component. Collectively, these findings highlight ZnGly as a promising holistic ingredient for comprehensive scalp and hair care formulations.

6. References

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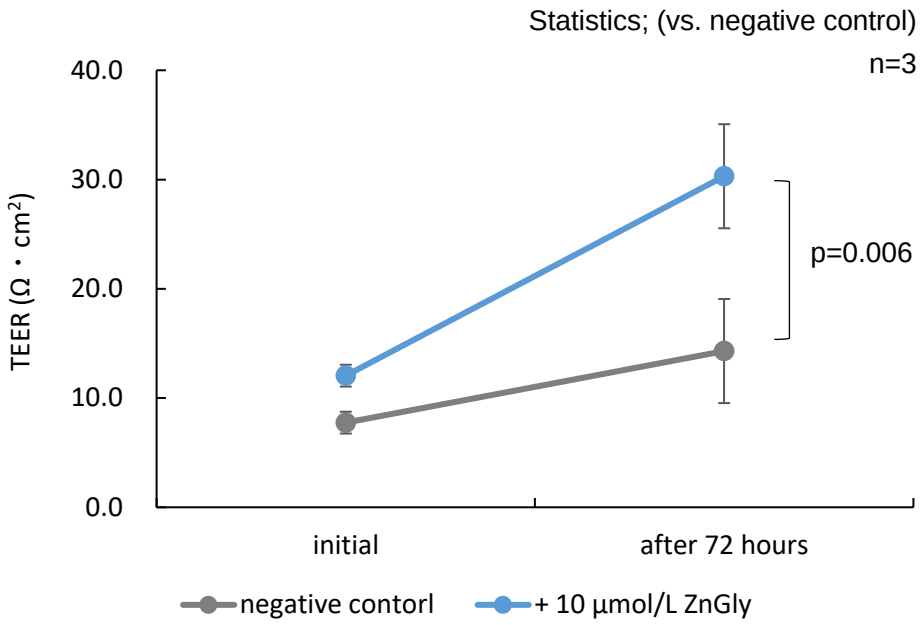

Figure 1. ZnGly induced barrier formation in keratinocytes. Transepithelial electrical resistance **(**TEER) was measured in cultured keratinocytes with and without ZnGly. TEER was increased by 10 µmol/L in the ZnGly treatment group than in the negative control. Statistical analysis was performed using Student's t-test.

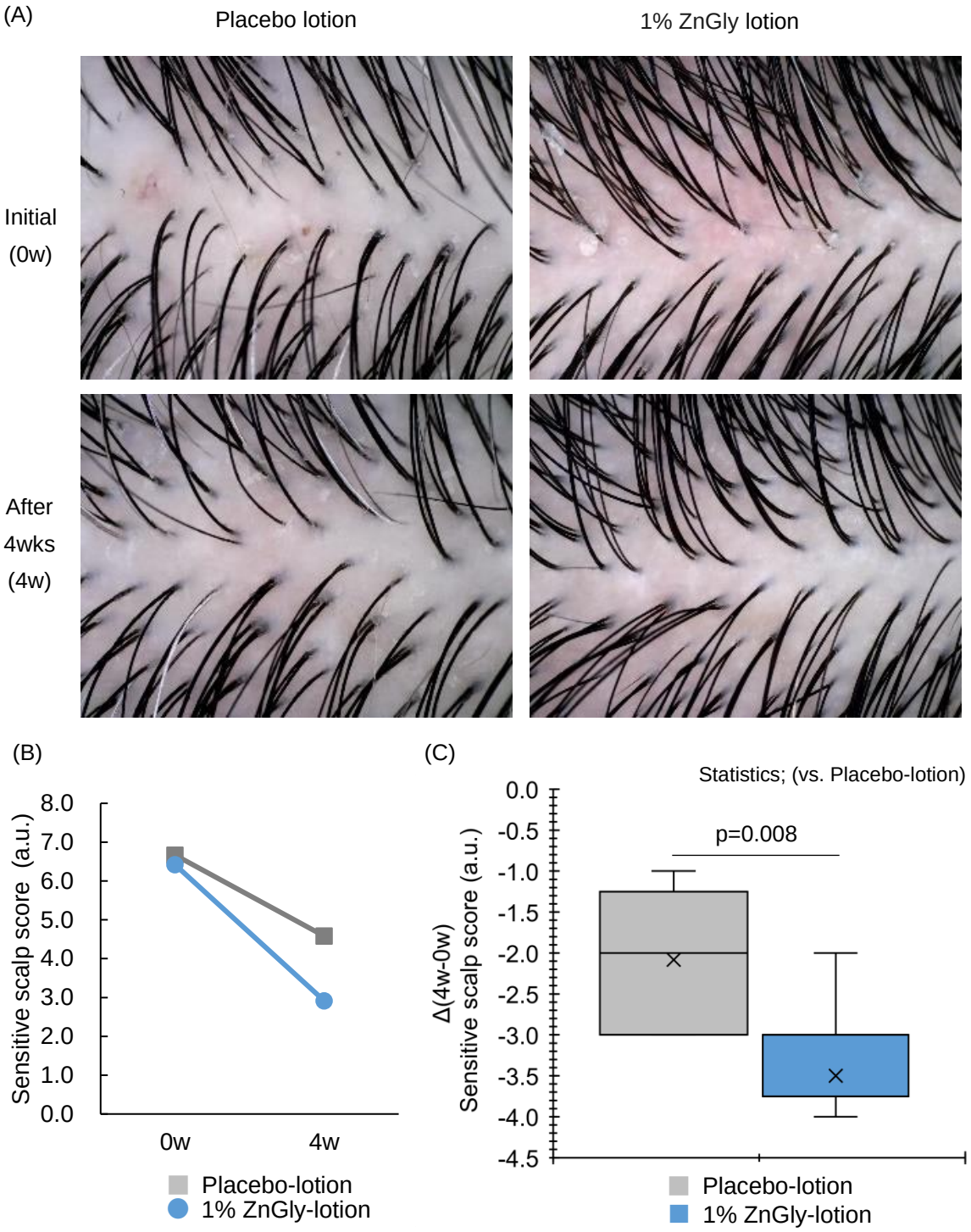

Figure 2. Topical application of ZnGly improve sensitive symptoms in scalp (A) Images of scalp condition at initial and 4 weeks of test lotion use; use of 1% ZnGly lotion resulted in better scalp redness and dandruff conditions than the placebo lotion. (B) Line plot showing sensitive scalp score mean value change over a period. Both lotion is effective for sensitive scalp. (C) Box plot of Δ(4w-0w) showing change in the amount subtracted graph of the sensitive scalp score; use of 1% ZnGly lotion significantly decreased the scalp scale compared to the placebo lotion. Statistical analysis was performed with the Mann Whitney U-test.

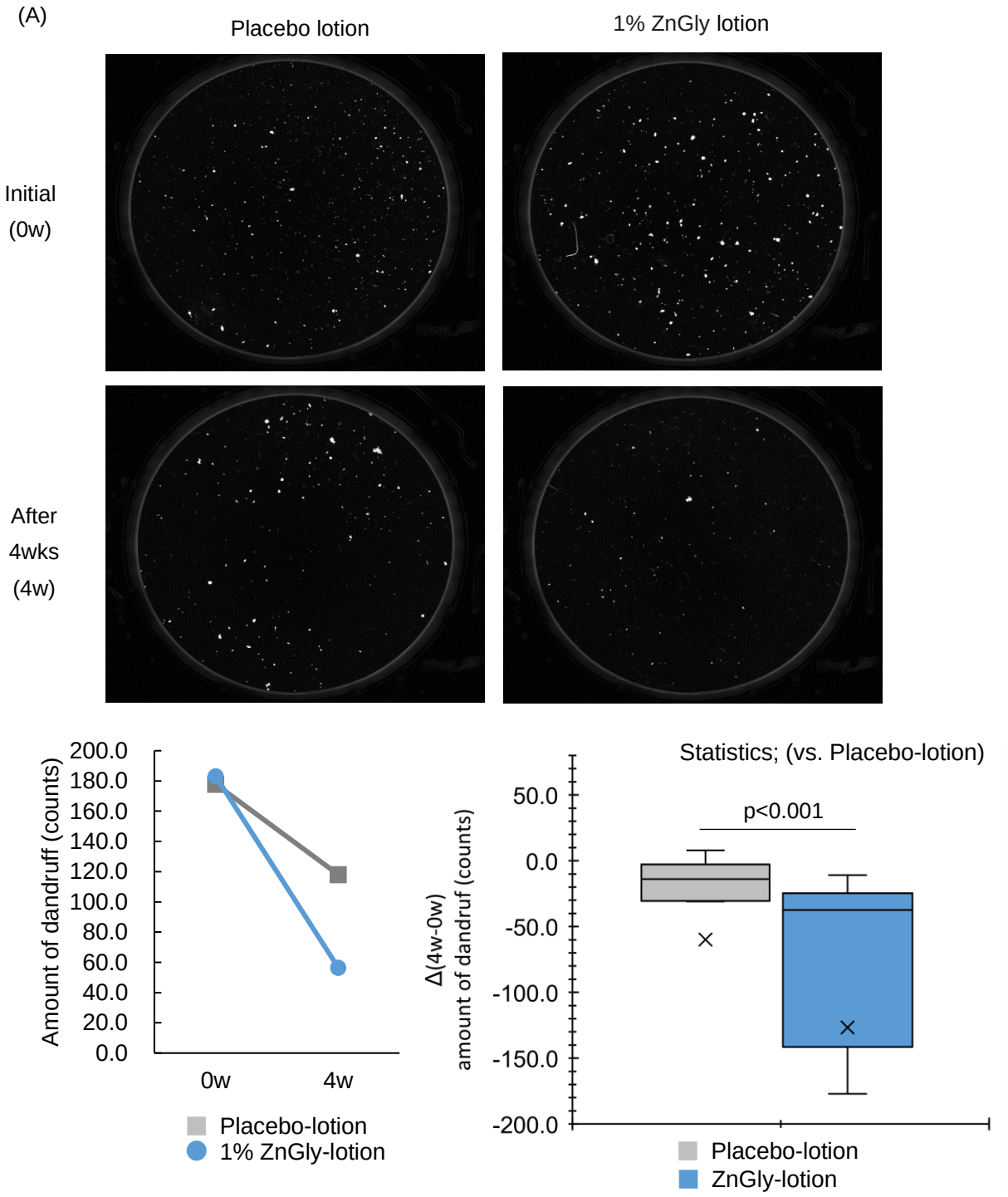

Figure 3. Topical application of ZnGly improves dandruff amount in sensitive scalps (A) The images of collected dandruff on a glass dish at initial and 4 weeks of ZnGly lotion use; use of 1% ZnGly lotion reduced the amount of dandruff. (B) Line plot showing the amount of dandruff mean value change over a period. Each lotion is effective for the amount of dandruff. (C) Box plot of Δ(4w-0w) showing change in the amount subtracted graph of the amount of dandruff; use of 1% ZnGly lotion significantly decreased the scalp scale compared to the placebo lotion. Statistical analysis was performed with ANCOVA.

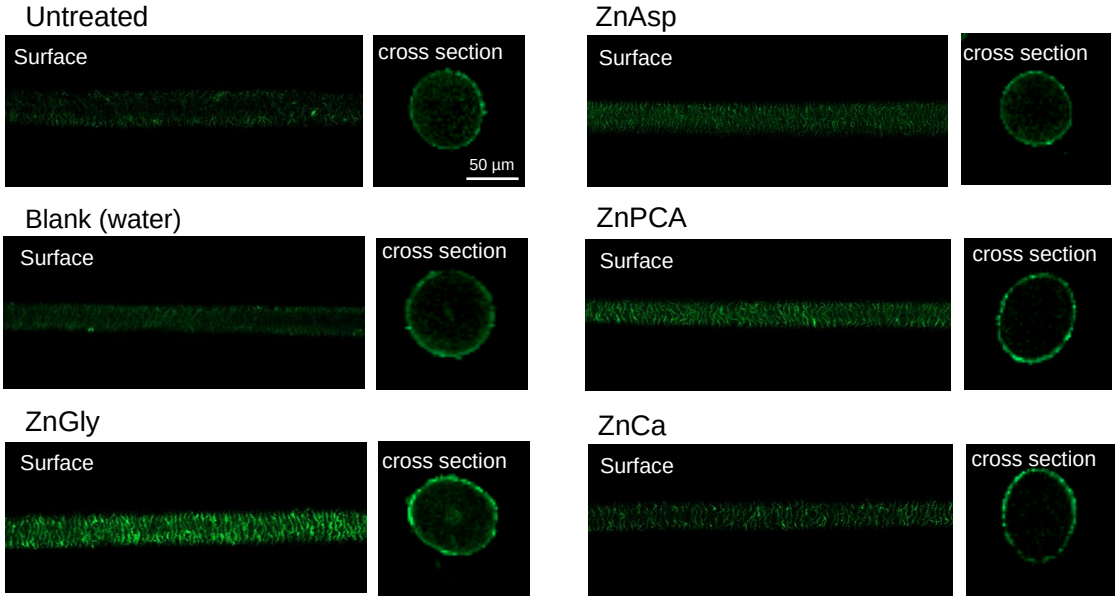

Figure 4. Fluorescent staining images of hair cuticle and cortex after treating by each Zn compound solution Strong fluorescence was observed in hair cuticle after treatment with ZnGly solution. In contrast, fluorescence in hair cortex did not change after treatment with various Zn compounds.