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Study on the Reparative Effect of Peptide RW3 Binding Active Collagen after Photoelectric Skincare

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Abstract

The photoelectric microneedle treatment instrument is also widely used due to the rapid development of medical cosmetology in China in recent years. It also causes a lot of skin discomfort after the consumers carry out such projects. This study, which combines small molecule active peptides (RW3) and active collagens and imitates the photoelectric treatment through *in vitro* and *in vivo* experiment, finds that small molecule active peptides (RW3) and active collagens have different improvement effects on cell proliferation, migration, anti-UV damage, inhibition of ear swelling and inflammation in mice, repair of UV damage and skin damage caused by microneedles.

Keywords

Photoelectric Microneedle, Treatment, Peptide, Collagen, Inflammation, Repair

1. Introduction

Microneedling, called as percutaneous collagen induction therapy, is a medical technology which a mechanical or physical, minimally invasive injury stimulation is made to the skin soft tissue with a micro needle instrument in order to obtain therapeutic or cosmetic effects; It can enhance the therapeutic or cosmetic effects by administrating drugs or functional ingredients synchronously or step-by-step, which the transdermal absorption efficiency is improving with the help of microneedles [1]. Microneedling has been proven to be suitable for improving skin problems such as skin aging, chloasma, acne, scar, striae atrophicae

and alopecia, obtaining a satisfactory therapeutic effect. Chun *et al.* analyzed research trends of microneedling in Korea in recent 10 years to provide basis for the future research and clinical application of microneedling [2].

Radiofrequency, a kind of microneedle technology, can cleavage, excise, fulgurate, ablate, electrocoagulate the tissues through electric heating. After RF energy at a specific temperature acts on the corium layer, it can make the tissue coagulate and collagen contract immediately and activate healing reaction to the secondary injury to achieve the therapeutic purpose of tissue remodeling. RF energy from the traditional RF instruments is easy to be absorbed by epidermal layer during the treatment. Loss of energy can not only cause the incomplete treatment on the skin lesions, but also damage healthy skin near the lesion, and increase the risk of complications such as pigmentation and scar after inflammation. To further improve the clinical therapeutic effect of radiofrequency and reduce the risk of adverse reactions, scholars combined the microneedle technology and RF energy to explore a new kind of minimally invasive radiofrequency therapy technology [3].

Most treatments get the therapeutic purpose of improving the striae atrophicae by stimulating the activity of fibroblasts directly or indirectly, inducing collagen synthesis and improving local skin type [4]. Saki et al recently published a single-center randomized controlled clinical trial (Comparison of the efficacy of microneedling versus CO_2 fractional laser to treat striae alba: A randomized clinical trial), which evaluates the efficacy of microneedling and CO_2 fractional laser in the treatment of striae atrophicae (white lines) [5]. However, if the operation is improper, CO_2 fractional laser can cause serious skin tissue damage, prolong patients' postoperative recovery time and cause poor treatment experience.

Peptide products have many applications in cosmetics such as repair, anti-wrinkle and whitening in recent years. Collagen, a kind of fibrous protein, is consistent with human connective tissue (mainly refers to skin, joints and bones). Due to the abundance, intensity and direct proportion to skin aging, collagen has attracted great attention in cosmetic industry. With the passage of time, collagen fibers are damaged, with a decreased thickness and intensity, which is closely related to skin aging [6].

As a product, the collagen is taken as the ingredient in different therapy regimens in cosmetic industry to make users become younger and healthier, which is often embodied in cream, bone and cartilage regeneration nutritional supplement, blood vessel and heart reconstruction nutritional supplement, skin regeneration nutritional supplement and skin softening nutritional supplement, etc [7].

At present, biomolecule can be extracted from natural sources such as plants and animals, or from the recombinant protein production systems, including yeast, bacteria, mammalian cells, insects or plants. The market scale of collagen will exceed USD 6.63 billion by 2025 due to its increasing use [8]. However, it is rare to use active collagen combined with peptide to repair skin redness, inflammation and other symptoms after cosmetic surgery with photoelectric equipment.

In this study, small molecule active peptides (RW3) combining with active collagen is used to make *in vitro* and *in vivo* experiments and MTT assay is used to determine the toxicity of different drug concentrations to WS1 and HaCat cells; Wound healing assay and Transwell test are used to detect the effect of drugs on cell migration; WB method is used to detect the expression of the cell stemness markers, CD44 and BMI, after the cells were treated with different drug concentrations; UVB preprocess method is used to detect the effect of drugs on reactive oxygen species and cell viability. The effects of photoelectric repair products on mouse allergic dermatitis induced by xylene, DRS540 microneedle roller and wound healing of mouse skin treated with UVB are studied by setting different dosages and administration time. By simulating the photoelectric treatment, it is found that small molecule active peptides (RW3) and collagens have different effects on cell proliferation, migration, anti-UV damage, inhibition of ear swelling and inflammation in mice, and repair of skin damage caused by microneedles.

2. Materials and Methods

2.1. Main Reagents and Instruments

The test samples are prepared by the laboratory itself. Sterile solution of effective components (for cell experiment): containing peptide RW3 and active collagen; Three-piece set of products for photoelectric post-surgery and sensitive skin care (for animal experiments): toner, ice mask, facial mask, and blank paste, Sichuan Liyangongfang Biotechnology Co., Ltd.

MTT (Thiazolyl Blue), Dimethyl sulfoxide (DMSO), SDS, polyacrylamide, Coomassie brilliant blue, tetramethylenediamine, β-mercaptoethanol, and Tween-20, Sigma-Aldrich (USA). Trypsin, Tris-base, glycine, and HE staining reagent, Beijing Suolaibao Technology Co. Ltd. (China) Fetal bovine serum, MEM-Alpha medium and PBS, Biological Industries (Israel). Protein standard (Marker), Thermo Fisher Scientific (USA). EDTA, biofroxx (Germany). PVDF membrane, Milipore (USA). CD44, Cell Signaling Technology (USA). BMI, GAPDH, Proteintech (China). Xylene, Chengdu Cologne Chemical Reagent Factory. Compound Dexamethasone Acetate Cream, Xinhecheng Holdings Group Co., Ltd. WNN Natural Skin Care Anti-Sensitive Moisturizing Tolerance-Extreme Cream (competitive product), purchased in the market.

HaCat cells (Human Immortalized Epidermal Cell), WS1 cells (Human Skin Fibroblast), both purchased from Cell Signaling Technology (USA). Kunming mice, SPF, 18 - 22 g, half male and half male, Chengdu Dashuo Experimental Animal Co., Ltd (China). Hairless mice, SPF, 11 - 12 weeks, half male and half male, Zhejiang Vital River Laboratory Animal Technology Co., Ltd.

Biosafety cabinet, Thermo Fisher Scientific (USA). Cell incubator, Thermo Fisher Scientific (USA). ULUPURE ultra-pure water manufacturing system, Sichuan ULUPURE Ultrapure Technology Co., Ltd. (China) Inverted Fluorescence Microscope, Olympus (Japan). High-speed refrigerated centrifuge, Ther-

mo Fisher Scientific (USA). Low speed centrifuge, Anhui Ustc Zonkia Scientific Instruments Co., Ltd. (China) Electric thermostatic water bath tank, Beijing Zhongxingweiye Instrument Co., Ltd. (China)

Ultrasonic cell crusher, Ningbo Scientz Biotechnology Co., Ltd. (China) Thermostatic metal bath, Haimen Kylin-Bell Lab Instruments Co., Ltd. (China) Full-automatic high-pressure steam autoclave, SANYO (Japan). Cell culture plate (6-well, 12-well and 96-well), Chengdu Ruiyin Biotechnology Co., Ltd. (China) Microplate reader, Molecular Devices (USA). Electronic analytical balance, Shanghai Tianmei Balance Instrument Co., Ltd. Pipette, Eppendorf (German). DRS540 microneedle roller (0.5 mm), Vitex (USA).

2.2. Method

2.2.1. Cell Proliferation/Toxicity Experiment - MTT Method [9] [10]

HaCat cells and WS1 cells in logarithmic phase were digested with trypsin to prepare cell suspension in 5×10^3 cells/ml, and cell suspension was inoculated into 96-well plate with 100 μL for each well, and cultured in the carbon dioxide incubator at 37˚C and 5% CO_2. After 24 h, the original culture solution was discarded, test samples with different concentrations were added, with 200 μL for each well, and 6 wells for each sample concentration in the test group and 6 wells in the blank group (200 μL cell culture medium for each well) were set. Incubation was continued for 24 h under the same conditions. 4 h before stopping the incubation, the 96-well plate was taken out and observed under the inverted microscope. 20 μl MTT solution of 5 mg/ml was added into each well, incubation was continued for 4 h, liquid in the well was discarded, 150 μl DMSO was added into each well, the plate was placed at room temperature for 10 min and oscillated on the thermostatic oscillator to make the solution color in the well uniform. Absorbance under the wavelength of 490 nm was determined with microplate reader.

2.2.2. Wound Healing Assay

HaCat cells and WS1 cells in logarithmic phase were digested with trypsin to prepare single-cell suspension in 1×10^7 cells/ml, which was inoculated into 6-well plate in 1×10^7 cells/well. After cultivating for 24 h, the original culture solution was discarded, two cross-shaped lines through the east, west, south and north were drawn at the center of each well in the 6-well plate with a 1 mL pipette tip, then cells were washed with MEM medium containing no serum for 3 - 5 times until the crossed cells were completely washed away during scribing. Each well was photoed under 10-fold microscope of inverted microscope. Then test samples with different concentrations were added into the wells of test group and cell culture medium was added into the wells of blank group. And each well was photoed again under 10-fold microscope of inverted microscope after 12 h. Cell migration distance was counted with Image software [11].

2.2.3. Transwell Experiment

MEM-Alpha containing 10% FBS was used to prepare test solutions with differ-

ent concentrations as the test group, and cell culture medium was used as blank group, and 800 μL of each group was added into the outer chamber respectively; HaCa cells and WS1 cells in logarithmic phase were digested with trypsin to prepare single-cell suspension in 3×10^5 cells/ml with MEN-Alpha medium containing no serum, which was inoculated into the inner chamber in 2×10^4 cells/well. After culturing in the incubator at 37˚C and 5% CO_2 for 24 h, chamber was taken out, supernatant was discarded, and chamber was washed twice with PBS solution and fixed with 4% paraformaldehyde for 20 min. After washing with PBS, crystal violet was added to dye for 30 min, then excess dye was washed away with PBS, and the cells and dyes on the inner wall of the inner chamber were swabed dry with cotton. The outer chamber was photoed under 40-fold microscope of inverted microscope after drying to observe the cells number in the outer chamber, and investigate the effect of test solutions with different concentrations on cell migration ability [11].

2.2.4. Western Blot Experiment

HaCat cells and WS1 cells in logarithmic phase were digested with trypsin to prepare 6mL single-cell suspension in 1×10^7 cells/ml, which was inoculated into the 5 CM^2 culture dish in 3×10^7 cells/3 mL. After being treated with cell culture medium (blank group) and test solutions with different concentrations (test group) respectively for 24 h, cells were collected, and 100 μL cell lysate was added with the cell concentration of 1×10^6 cells/ml. The dish was oscillated and put on the ice for 40 min, then was centrifuged for 12 min at 4˚C in 13,000 rpm. The protein sample in supernatant was quantified by the method of bicinchoninic acid (BCA kit), sample buffer was added and mixed evenly, then denatured by heating at 95˚C for 10 min [11] [12]. After separation by 12% SDS-PAGE electrophoresis, the membrane was transferred to PVDF membrane. Primary antibody was added respectively (The dilution ratio of primary antibody is 1:1000), and the membrane was incubated at 4˚C overnight. The membrane was washed with TBST, and incubated with the second antibody marked by HRP (1:6000) at 37˚C for 1 h. Then PVDF membrane was washed by TBST for 5 times, 10 min for each time. Then the expression of each protein was detected by ECL chemiluminescence to investigate the effects of effective components on the expression of stemness-related proteins (CD44 and BMI) in Hacat cells and WS1 cells [13].

2.2.5. UVB Irradiation Experiment

Cells were inoculated into 96-well plate in 5×10^3 cells/well, cultured in incubator at 37˚C, 5% CO_2 and saturation humidity for 24 h, then divided into 5 groups, with 6 wells for each group: One group was added with cell culture medium and not irradiated, one group was added with cell culture medium and irradiated, and three test groups were added with test solution with different concentrations and irradiated. All the irradiation groups were irradiated by UVB at 40 mJ/cm^2 [14], 100 μL medium was added to each well after irradiation and continued to be cultured in the incubator for 24 hours, and the cell viability was detected by MTT method [15] [16] [17].

2.2.6. ROS Detection Experiment

According to the kit method, DCFH-DA was diluted with medium containing no FBS and protein at 1:1000 [13] to make the final concentration 10 um/L. The cells treated with test solution and UVB irradiation were suspended in the diluted DCFH-DA and incubated in incubator for 20 min at 37˚C. The cells were turned upside down and mixed every 3 - 5 times to make them fully contact with the probe. The cells were washed with medium containing no FBS and protein for 3 times to sufficiently remove DCFH-DA which does not enter cells. Fluorescence was detected by flow cytometry [18].

2.2.7. Experiment on Ear Swelling Induced by Xylene in Mice [19] [20]

After adaptive feeding for 3 days, 60 Kunming mice were divided into 6 groups randomly, 10 mice in each group. The blank group was applied with normal saline, 0.2 g/mouse; the control group was applied with blank paste without effective components, 0.2 g/mouse; Groups with low-, medium- and high-dose were applied with test sample, 0.1 g/mouse, 0.2 g/mouse and 0.4 g/mouse; Positive control group was applied with Compound Dexamethasone Acetate Cream, 0.2 g/mouse. The reagents and drugs were applied on both sides of the mice's right auricle, and the left ear was not applied as control, with 7 days of continuous administration. At 1 hour after the last administration, 30 μL xylene was applied on both sides of the mice's right auricle in each group to cause inflammation. After 30 min of inflammation, the mice were killed and the left and right ears were cut off along the auricle baseline. Round ear pieces with the same area in both ears were punched with 6mm puncher and weighed with analytical electronic balance, and auricle swelling degree and inhibition rate (%) were calculated with the following formula.

Swelling degree (mg) = weight of right ear piece (mg) − weight of left ear piece (mg)

Swelling inhibition rate (%) = (mean swelling degree of blank group − mean swelling degree of administration group)/mean swelling degree of blank group × 100%

2.2.8. Experiment on Skin Damage Caused by Roller Microneedle

After adaptive feeding for 3 days, 12 inbred hairless mice were divided into 6 groups randomly, 2 mice in each group. Combined with the results of pre- experiment on skin damage modeling caused by roller microneedles, the back of each mouse was rolled for 4min with German DRS540 microneedle roller microneedle (0.5 mm) to cause skin damage, and the blank group was applied with normal saline, 0.2 g/cm^2/mouse; The control group was applied with blank paste without effective components, 0.2 g/cm^2/mouse; Groups with low-, medium- and high-dose were applied with test sample in 0.1 g/cm^2/mouse, 0.2 g/cm^2/mouse and 0.4 g/cm^2/mouse; Positive control group was applied with WNN Natural Skin Care Anti-Sensitive Moisturizing Tolerance-Extreme Cream, 0.2 g/cm^2/mouse. The skin damage on the back of mice in each group was applied continuously for 4 days, once a day. Mice were killed after 4 days, the

treated skin was collected and the enrichment of inflammatory cell was observed with the H&E staining [21] [22] [23].

2.2.9. Experiment on Skin Damage Induced by UVB

After adaptive feeding for 3 days, 12 inbred hairless mice were divided into 6 groups randomly, 2 mice in each group. Combined with the results of pre-experiment on skin damage modeling caused by UVB, the back of each mouse was irradiated with UVB of 110 mJ/cm^2 to cause skin damage, and the blank group was applied with normal saline, 0.2 g/cm^2/mouse; The control group was applied with blank paste without effective components, 0.2 g/cm^2/mouse; Groups with low-, medium- and high-dose were applied with test sample in 0.1 g/cm^2/mouse, 0.2 g/cm^2/mouse and 0.4 g/cm^2/mouse; Positive control group was applied with WNN Natural Skin Care Anti-Sensitive Moisturizing Tolerance-Extreme Cream, 0.2 g/cm^2/mouse. The skin damage on the back of mice in each group was applied continuously for 4 days, once a day. Mice were killed after 4 days, the treated skin was collected and the enrichment of inflammatory cell was observed with the H&E staining [24] [25].

3. Result and Analysis

3.1. Cell Proliferation/Toxicity Experiment

The cytotoxicity of test solution containing effective components on HaCat and WS1 cells was detected by MTT method. We can see from the **Figure 1** that after the two kinds of cells were interacted with test solutions with different concentrations for 24 h, the 6-fold dilution of the test solution was not toxic to the two kinds of cells, of which the cell activity is about 5 times that of the control group under this concentration, indicating that the test solutions with this concentration have obvious enhancement and promotion effects on proliferation and regeneration ability of both kinds of cells [10].

3.2. Wound Healing Assay

Wound healing assay is the most commonly used model to detect wound healing *in vitro*, which effectively simulates the process of cell stretching, proliferation, migration and covering the wound surface in the early stage of wound healing [26] [27]. This model was selected to measure the width of scratch blank area at 0h after scratching and 12 h after treating with test samples respectively, and the difference between them was calculated to evaluate the effect of effective components on the migration of HaCat and WS1 cells. See **Figure 2** and **Figure 3** for the results. When the two kinds of cells were treated with test solutions of different concentrations, cells all proliferated to the scratches at different degrees at 12 h after scratching, among which the effect of 6-fold dilution of test solution is the most obvious, showing that the concentration of effective components could improve the cell proliferation and regeneration ability, and promote wound healing and repair [28].

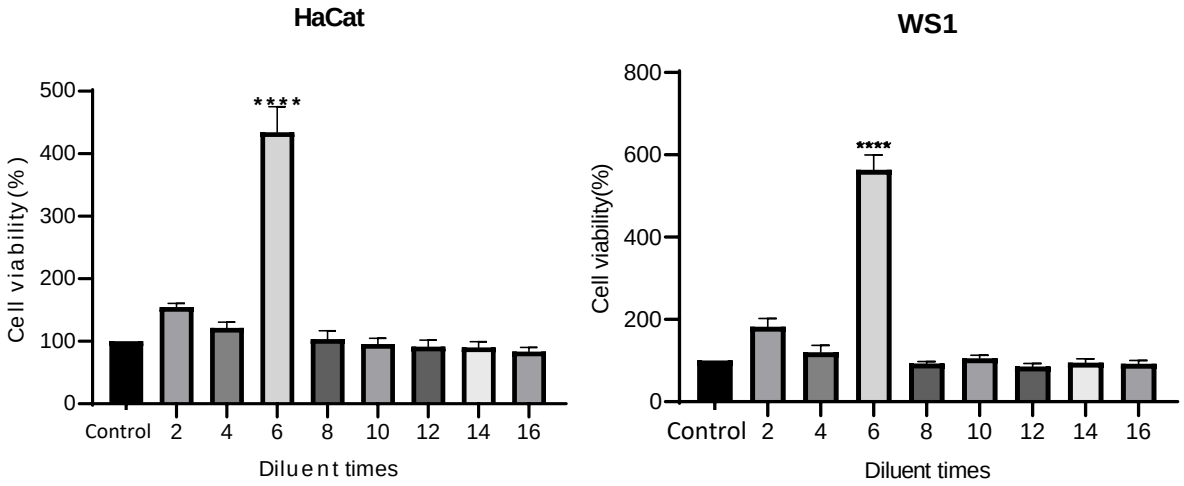

Figure 1. Effects of effective components on cell proliferation/toxicity of HaCat cells and WS1 cells. (Note: compared with the control group, ****means P < 0.0001.)

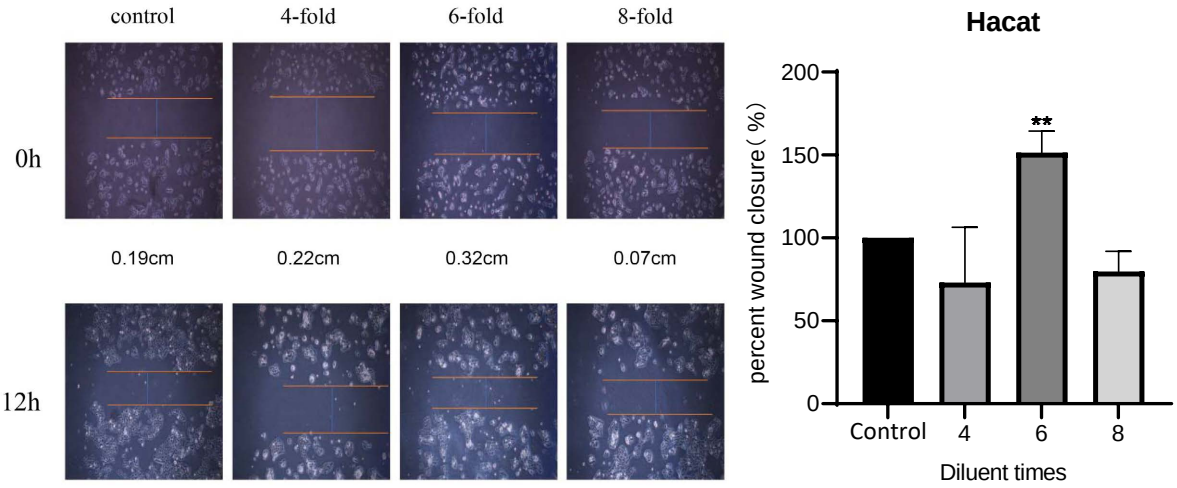

Figure 2. Effects of effective components on HaCat cells migration studied in wound healing assay. (Note: compared with the control group, **means P < 0.01.)

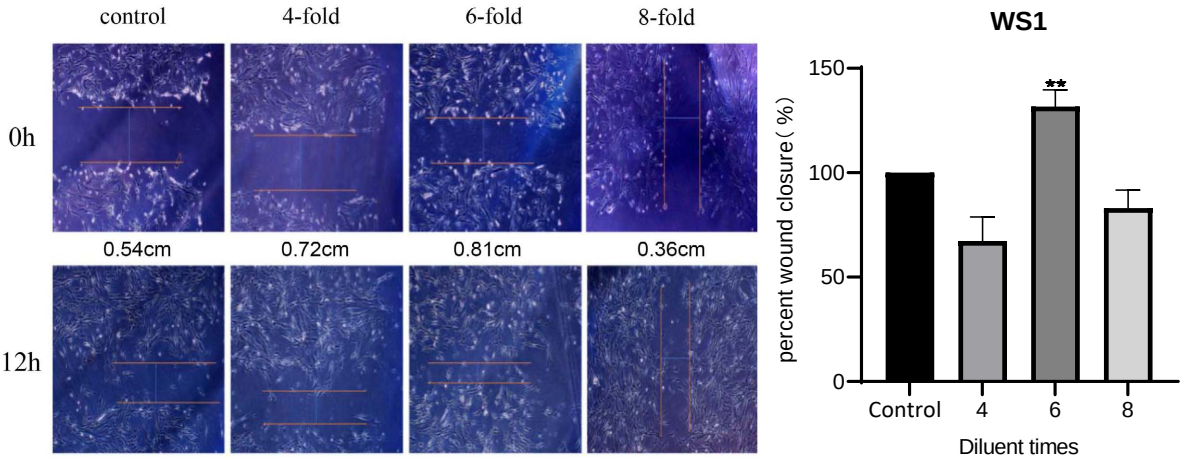

Figure 3. Effects of effective components on WS1 cells migration studied in wound healing assay. (Note: compared with the control group, **means P < 0.01.)

3.3. Transwell Experiment

See Figure 4 and Figure 5 for the experiment results. The results of 3 experiments showed that the number of membrane passed though by HaCat and WS1 cells increased in different degrees after adding effective components, compared with the control group (C) without effective component, confirming that the effective components enhanced the migration ability of HACAT and WS1 cells [11], especially the 6-fold dilution of the test samples was the best.

3.4. Western Blot Experiment

After the two kinds of cells in test group were treated with effective components with different dilution times, the expression of stemness-related proteins BMI and CD44 in cells increased compared with the cells in blank group (C) without treatment, and the increase was obvious at the 6-fold and 8-fold dilution, as shown in the Figure 6 and Figure 7. It shows that the effective components can enhance the stemness of two kinds of cells and promote the renewal and differentiation of cells [29].

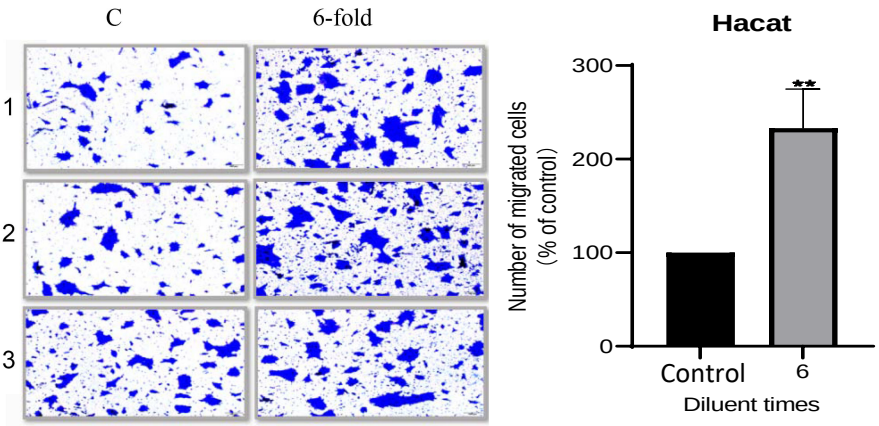

Figure 4. Effects of effective components on HaCat cells migration. (Note: compared with the control group, **means P < 0.01.)

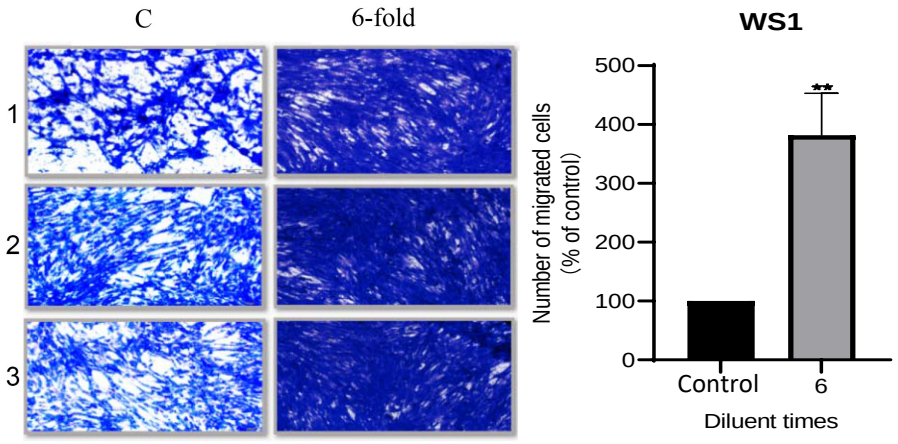

Figure 5. Effects of effective components on WS1 cells migration. (Note: compared with the control group, **means P < 0.01.)

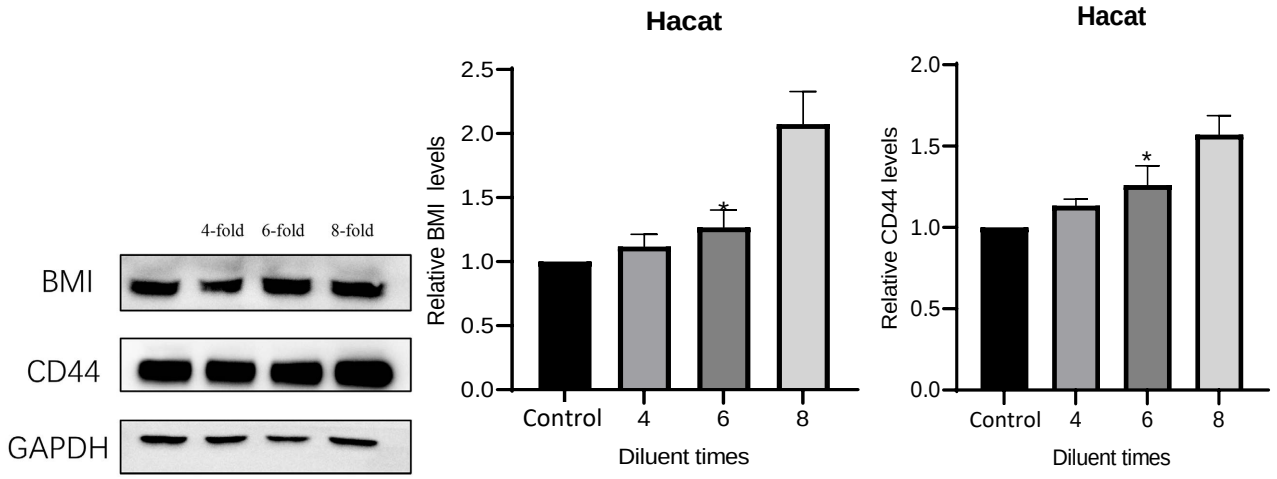

Figure 6. Effects of effective components on BMI and CD44 of HaCat cells. (Note: compared with the control group, *means $P < 0.05$.)

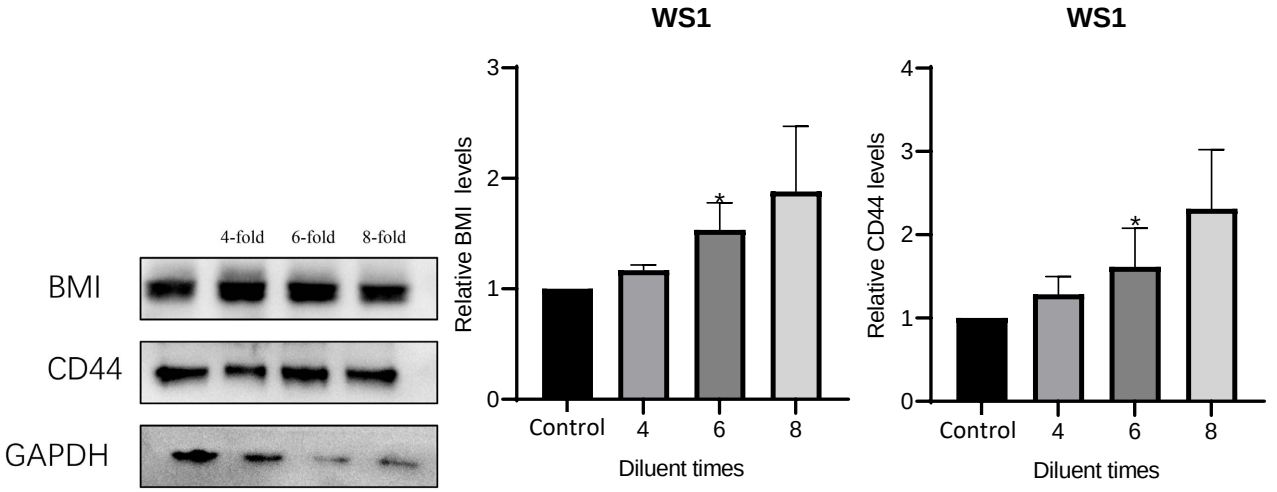

Figure 7. Effects of effective components on BMI and CD44 of WS1 cells. (Note: compared with the control group, *means $P < 0.05$.)

3.5. UVB Irradiation Experiment

The protective effects of effective components on UVB light damage of two kinds of cells were evaluated *in vitro*, and the experimental results were shown in **Figure 8**. After being irradiated by UVB of 40 mJ/cm^2, the survival rate of the two kinds of cells decreased. But the survival rate of cells treated with effective components with different concentrations increased after UVB irradiation, especially for HaCat cells, the effect is more significant. It showed that proper concentration of effective components can effectively repair skin cells and protect skin cells from damage caused by UVB [30].

3.6. ROS Detection Experiment

Skin damage was closely related to the increase of reactive oxygen species (ROS) in skin cells [31] [32], UVA and UVB in UV can cause the oxidative stress reac-

tion in skin cells to increase and generate ROS [33], make damage to biological macromolecules such as protein, nucleic acid and lipid, and affect its normal physiological and biochemical functions, causing the cell necrosis or apoptosis [34] and skin photoaging [35]. ROS kit was used to detect the ROS levels of cells treated with and without effective components after UVB irradiation respectively. The experiment results in the **Figure 9** showed that the 6-fold and 8-fold dilutions of the test solution could effectively eliminate ROS in WS1 cells, further confirming the protective effect of the effective components of this concentration on UVB light damage of the cells. For HaCat cells, the ROS level in cells treated with 6-fold dilution of the test solution was not significantly different from that of the control group.

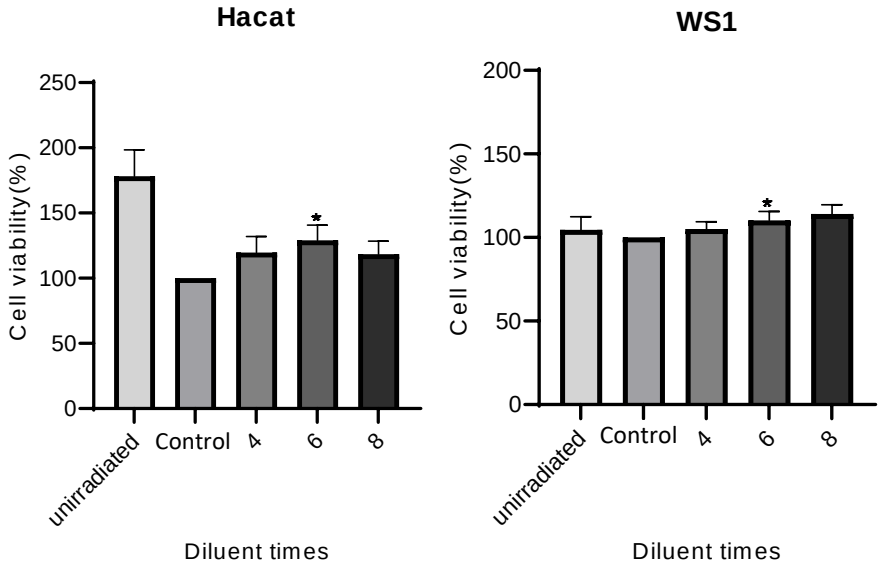

Figure 8. Protection of effective components on UVB light damage of HaCat cells and WS1 cells. (Note: compared with the control group, *means $P < 0.05$.)

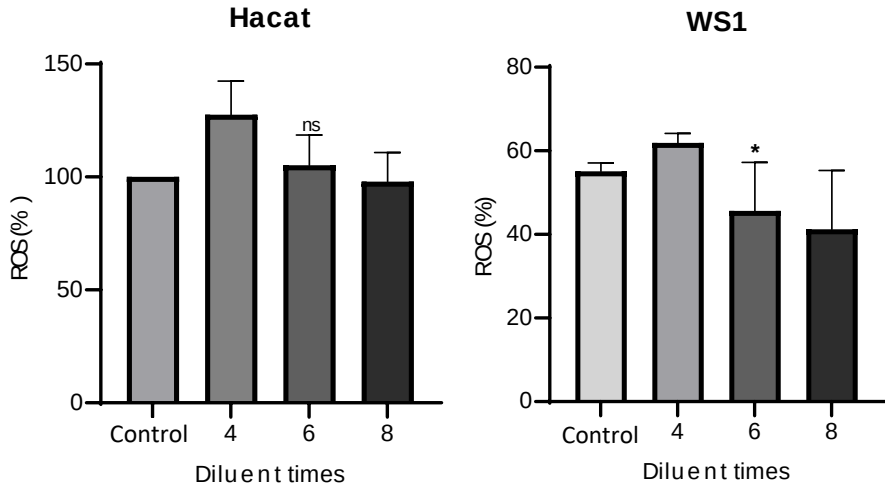

Figure 9. Effect of effective components on ROS level in HaCat and WS1 cells after UVB irradiation. (Note: compared with the control group, *means $P < 0.05$.)

3.7. Effect of Effective Components on Mice's Ear Swelling Induced by Xylene

The effects of different doses of test samples on acute inflammation in mice were studied with anti-inflammatory experiment with mouse ear piece method. The test samples were applied on the front and back side of the right auricle of mice for 7 days consecutively, and no obvious irritation to the skin of mice was found. The ear swelling degree and its inhibition rate of mice in each group were shown in **Figure 10** and **Figure 11** respectively. Compared with blank model group, positive group could significantly inhibit the auricle swelling degree of mice, and the inhibition rate reached 81.1%. However, the low, medium and high dose groups of the test samples showed different inhibition functions on mice ear swelling, among which the medium dose group had the most significant inhibition effect, with the inhibition rate of 47.1%, being 58.1% of that of the positive group. The blank paste without effective components had no inhibitory effect on mice's auricle swelling. The experimental results showed that effective components with a certain concentration have an obvious inhibitory effect on acute inflammation [20].

3.8. Experiment on Skin Damage Caused by Roller Microneedle

Skin damage and inflammation on the back of hairless mice were caused by DRS540 microneedle roller microneedle (0.5 mm), and different doses of test samples were applied to the skin damage and inflammation to investigate the therapeutic effect of the tested samples on allergic inflammation of skin and protein fiber. The results are shown in **Figure 12**. Compared with the blank group applied with normal saline and control group applied with blank paste, the skin pathological damages treated with test samples of low-, medium- and high-dose were obviously alleviated, in which the infiltration of inflammatory cells dominated by neutrophils was obviously improved, and the high-dose group had the best anti-inflammatory effect, which was equivalent to or even exceeded that of the positive control group, showing that the test sample had excellent anti-sensitive, anti-inflammatory and skin damage repair effects.

3.9. Experiment on Skin Damage Induced by UVB

Ultraviolet light (UVA, UVB and UVC) is the most important environmental factor causing skin aging, among which UVB radiation can generate too much reactive oxygen species in the body [15], which breaks the dynamic balance between the generation and elimination of reactive oxygen species, and causes the oxidative stress of cells, and promotes the cell viability to decrease or even die [17]. Long-term ultraviolet radiation can lead to skin darkening, sunburn, skin inflammation diseases and even skin cancer [36]. In this experiment, hairless mice's back skin damaged by UVB irradiation was treated with test samples of different doses, and the results of HE staining of skin tissue were shown in **Figure 13**. After being treated with test samples with low-, medium- and high-dose,

the pathological damages of damaged skin were obviously alleviated and inflammation was slight compared with blank group and control group. Among the low-, medium- and high-dose groups, the high-dose group has the best effect, which is equivalent to or even better than that of the positive control group, showing that the test samples containing effective components have relieving and repairing effects on skin inflammation and injury induced by UVB.

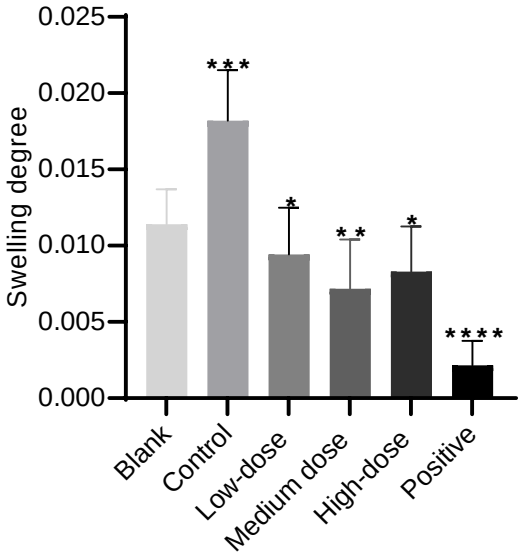

Figure 10. Ear swelling degree of mice in each group. (Note: compared with the control group, *means P < 0.05, **means P < 0.01, ***means P < 0.001, ****means P < 0.0001.)

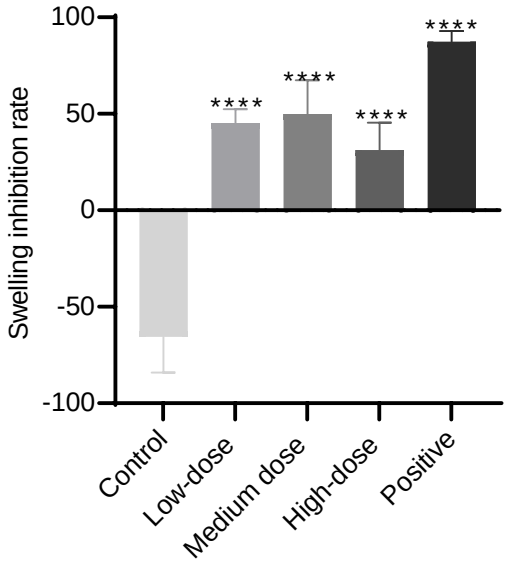

Figure 11. Ear swelling inhibition rate of mice in each group. (Note: compared with the control group, *means P < 0.05, **means P < 0.01, ***means P < 0.001, ****means P < 0.0001.)

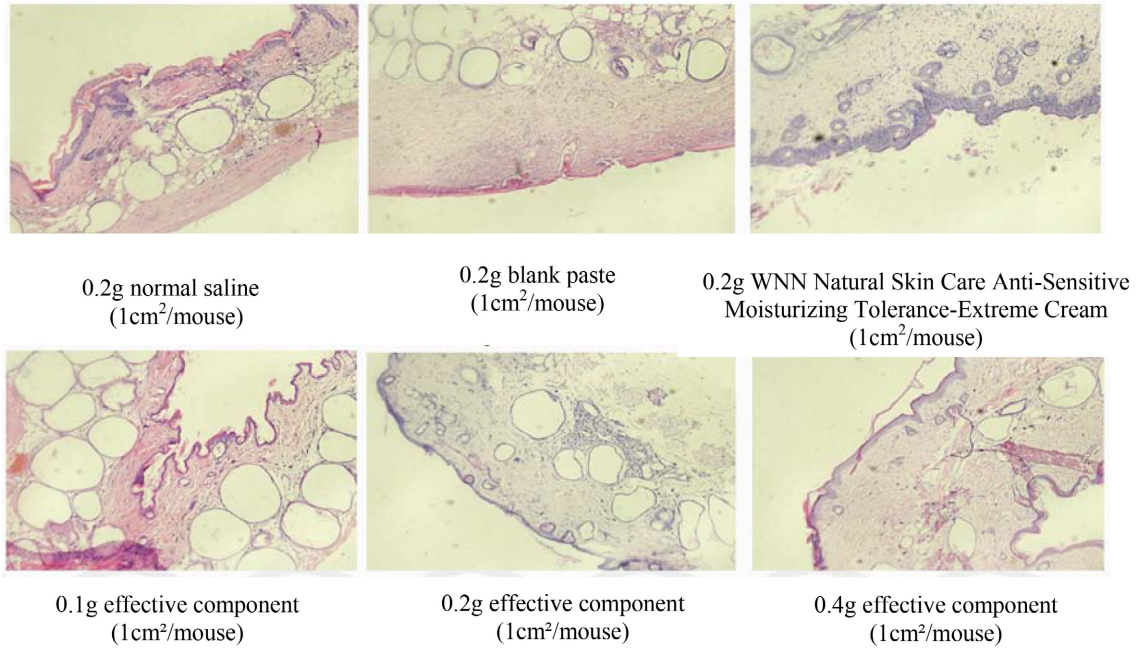

Figure 12. HE staining of mouse back skin after treating with roller.

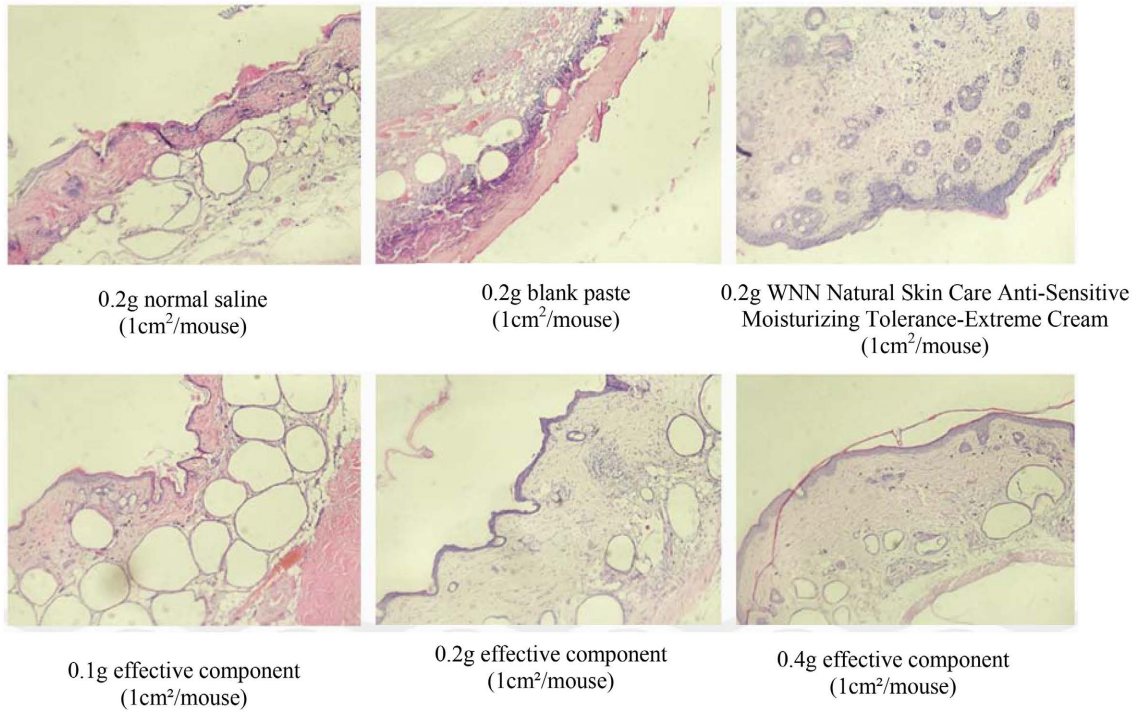

Figure 13. HE staining of mouse back skin after UVB irradiation.

4. Conclusion

The results of the above cell experiment and animal experiment show that after 6-fold dilution, the samples of peptide RW3 and active collagen composition made in our laboratory are non-toxic to cells, and can obviously promote the proliferation, differentiation and regeneration of the two kinds of cells ($P <$

0.001); At the same time, the composition sample can effectively remove ROS in HaCat and WS1 cells, repair skin cells and reduce the light damage caused by UVB. Three-piece set of products containing peptide RW3 and active collagen made in laboratory, namely toner, ice mask and facial mask, were mixed as 1:1:1 for animal experiments. The results of experiment on ear swelling induced by xylene in mice show that the medium dose group of this product can significantly inhibit the mice's ear swelling caused by xylene, and has the effect of anti-acute inflammation; Hairless mice skin was treated by simulating roller microneedles, photoelectric beauty technology and UVB irradiation, and test product was applied on the wound skin. The results of HE staining showed that the test samples had excellent anti-sensitive, anti-inflammatory and skin damage repair effects.

5. Discussion

With the development of science and technology, microneedle gets great progress and breakthrough achievements in beauty and skin care field, which application is mainly to deliver active substances for beauty, combine with electricity, light and heat for beauty, and combine with radiofrequency for reducing skin acne and filling skin to achieve rejuvenation, cleaning skin, hair transplantation and so on [37]. Microneedle radiofrequency (MRF), combining microneedles, radiofrequency and fractional technology, is used clinically to treat skin aging, acne vulgaris and its related skin lesions, striae atrophicae, etc., which the effectiveness has been widely confirmed [38]. But at the same time, microneedle will also bring some adverse reaction to users' skin, such as local pain, bleeding, skin dysfunction (edema, erythema, edema, etc.). And chronic skin wounds are prone to a long inflammation reaction period, and even some people have reactive acne, post-inflammatory pigmentation, allergy and hypersensitivity after using microneedles [39] [40] [41] [42]. For the postoperative care of microneedling, it generally takes measures within 7 days after operation, such as making a cold compress with mask, making an irradiation with LED, making an external applying with medical moisturizing masks and moisture burn ointment, making an external use of repair products of epidermal growth promoting factor and so on; It should be moisturized and protected from the sun strictly at 1 - 2 weeks after surgery [41]. Collagen is a very popular effective component in skin repair products. Many collagen peptides are related to the growth, differentiation, division, proliferation and migration of skin cells, which can provide skin nutrients, delay skin aging, promote skin wound repair, and have the functions of moisturizing and anti-radiation [43]. Combining with the action mechanism of microneedle, it takes active intervention measures to supplement collagen, promote skin cell proliferation, repair skin damage, accelerate wound healing, and offset the side effects and adverse reactions caused by microneedles to the greatest extent in the three main stages of wound healing cascade reaction, namely inflammation stage, proliferation stage and remodeling stage [40] [44].

Based on this purpose, this study used small molecular active peptide (RW3) combined with active collagen to carry out *in vivo* and *in vitro* experiments, and simulated microneedle, photoelectric beauty technology and UVB irradiation to treat with mice skin. The results showed that small molecular active peptide (RW3) and active collagen had positive effects on promoting cell proliferation, preventing ultraviolet damage, inhibiting ear swelling and inflammation in mice, and repairing skin damage caused by microneedles, which proves that it is feasible to use small molecule active peptide (RW3) combined with active collagen to repair skin damage wounds after microneedle and photoelectric beauty surgery, but the reaction and efficacy of its application in human still need to be further studied.

Ethical Approval

The experiment was carried out in accordance with the guide on the care and use of animals during the experiment from the Ethics Committee of the Animal Center, Chengdu Medical College.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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