ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Prevention and Repair of Ultraviolet B-Induced Skin Damage in Hairless Mice via Transdermal Delivery of Growth Factors Immobilized in a Gel-in-Oil Nanoemulsion

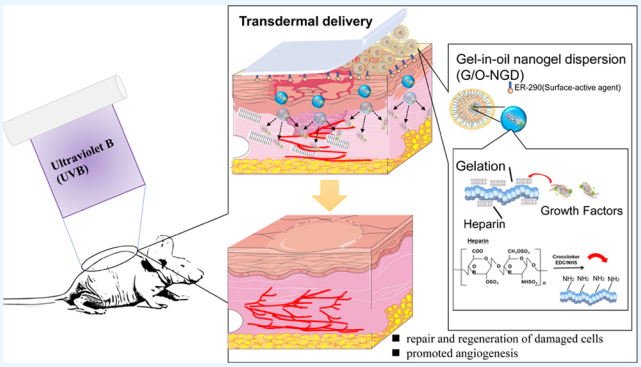
Yi Zhang, Yuuta Inoue, Jannatul Fardous, Ryota Doi, Takahiro Ijima, Toshioh Fujibuchi, Yo-ichi Yamashita, Shinichi Aishima, and Hiroyuki Ijima*

Cite This: *ACS Omega* 2023, 8, 9239−9249

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Ultraviolet (UV) radiation from the sun or artificial sources is one of the primary causes of skin damage, including sunburns, tanning, erythema, and skin cancer. Among the three different types of UV rays, UVB rays have a medium wavelength that can penetrate the epidermal layer of the skin, resulting in sunburn, suntan, blistering, and melanoma in case of chronic exposure. This study aimed to evaluate the preventive and therapeutic effects of a gel-in-oil nanogel dispersion (G/O-NGD) as a transdermal delivery biomolecular carrier for skin damage caused by UVB light. The efficacy of this carrier against UVB-induced skin damage was investigated in vivo by delivering different growth factors (GFs) encapsulated in a G/O-NGD. Artificial UVB light was used to induce skin damage in nude mice, followed by the transdermal application of five GF [vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), transforming growth factor (TGF)-1, and insulin-like growth factor (IGF)-α]-immobilized G/O-NGD. Among these GFs, VEGF and bFGF promoted angiogenesis, while EGF, TGF-1, and IGF-α promoted the repair and regeneration of damaged cells. The results showed that G/O-NGD was superior to heparin-immobilized G/O-NGD in reducing UVB-induced skin damage, such as erythema, epidermal water reduction, inflammation, and dermis thickening. In addition, G/O-NGD could prevent and treat abnormal follicle proliferation caused by UVB rays and exhibited potential to repair lipid glands. Overall, our results demonstrate the potential of G/O-NGDs for the treatment of UVB-induced skin damage.



1. INTRODUCTION

The spectrum of the sun emits electromagnetic waves. The atmosphere and ozone layer filter away the majority of hazardous high-energy radiations; however, the ultraviolet (UV) rays (200−400 nm) can reach the Earth and even pierce human skin.[1] Solar UV radiation consists of 1−10% high-energy medium-wave UVB (290−320 nm) and 90−99% long-wave UVA (320−400 nm) rays,[2] which are completely absorbed by the stratospheric ozone. Upon sunlight exposure, high-energy (mutagenic) UV light with a wavelength (WL) of 300 nm is nearly completely absorbed by the epidermal cell components, while low-energy UV light with a high WL penetrates deeper into the skin.[3] UV radiation is responsible for almost all adverse effects of sunlight on humans, including sunburn, photodamage to the dermal connective tissue, photoaging, and erythema. Notably, exposure to UV radiation is the leading cause of skin cancer in humans.[4] UVB is the most powerful type of solar radiation that reaches the Earth's surface and causes oxidative stress.[2,5] Its adverse effects are mainly caused by DNA and protein damage, leading to cell cycle arrest and apoptosis.[6] Aromatic heterocyclic bases present in DNA and RNA have absorption maxima at a WL of 260−265 nm, which is close to the WL of UVB light. Direct UVB absorption causes pyrimidine bases to dimerize, and replication of damaged DNA causes nucleotide misincorporation at photopermeable locations, resulting in mutations. UVB-induced mutations cause distinct changes in DNA, making UVB an effective tumor promoter.[2,7] Therefore, the development of effective strategies to decrease UVB-induced keratin-forming cell damage is essential.

In recent years, drug delivery through the skin to combat UVB damage has been an area of high interest. Reduced first-pass metabolism and systemic side effects are the advantages of transdermal delivery. A relatively low dose of the drug can

Received: November 16, 2022
Accepted: February 23, 2023
Published: March 3, 2023

reduce treatment costs and improve patient adherence through an effective sustained-release effect.[8,9] New nanoemulsion carriers can significantly reduce the skin irritation caused by transdermal drug delivery. The benefits of nanoemulsions include epidermal hydration to achieve moisturization, improvement of the skin's protective barrier and sustained drug release. Therefore, nanoemulsions can be used to improve the bioavailability and efficacy of drugs by preventing drug accumulation in the skin.[10−13]

Growth factors (GFs) act as biomacromolecular drugs because of their effects on cell growth, tissue regeneration, and angiogenesis.[14−16] The basic fibroblast growth factor (bFGF) promotes skin ulcer healing by inducing fibroblast proliferation. Although the biological activity of the growth factor is brief, it takes a relatively long time to cause the desired effect, making it difficult to maintain an effective local concentration over time.[17−19] Therefore, it is necessary to create a drug carrier to increase the stability of the encapsulated drug and break the barrier function of the outermost layer of the skin, followed by sustained drug effects in the body. Heparin can be immobilized on biodegradable collagen (Hep-col) or gelatin (Hep-gel) by chemical cross-linking of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/*N*-hydroxysuccinimide (NHS).[20] Since heparin has a negative charge due to the large number of sulfate groups within its molecular structure, it prevents the thermal expansion of GFs by electrophilic binding to positively charged GF. Thereby, the inner phase of the gel-in-oil nanogel dispersion (G/O-NGD) formed of hep-gel, improving their stability within the body.[21] In previous studies of our laboratory, GFs were added to hep-col films, and the loading rate of GF was determined by the ELISA method, which was about 80−90%. Moreover, through HUVEC proliferation, it was found that the hep-col membrane during preincubation could stabilize bFGF and maintain its biological activity for about twice as long as the bFGF solution. The slow-release effect of hep-gel on GF was also verified.[22]

In this study, we further developed our recently reported gel-in-oil nanogel dispersion (G/O-NGD), which showed stable physicochemical properties and storage stability. In vitro skin permeation studies indicate that fluorescent proteins containing G/O emulsions have better penetrability through the stratum corneum than GF aqueous solutions. In vivo studies have revealed that GF-immobilized G/O emulsions have an angiogenesis-inducing effect after transdermal application.[23] As a result, G/O emulsions can be utilized effectively to distribute GFs to the target spot while maintaining good stability. Heparin can not only immobilize foreign proliferative factors but may also trigger the activity of native proliferative factors. G/O emulsions can prevent the immobilization of proliferative factors.[24]

UVB is an established cause of skin cancer because of its ability to cause DNA damage in skin cells, which, if not repaired effectively, may eventually lead to the accumulation of oncogenic mutations, resulting in skin cell malignancies.[25] As a result, prevention of UVB damage and post-irradiation repair are essential. Similarly, the supply of nutrients from the body to the damaged site via angiogenesis and the repair and regeneration of damaged cells are important factors for wound healing.

Considering the above circumstances, this study is aimed to develop a novel drug delivery system for transdermal delivery of growth factors (GFs) to repair ultraviolet (UV) ray-mediated skin damage. Different GFs, namely, the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which promote angiogenesis, and the epidermal growth factor (EGF), transforming growth factor (TGF)-1, and insulin-like growth factor (IGF)-α, which promote the repair of damaged cells are delivered via immobilization in G/O-NGD. At the same time, the efficacy of GF-loaded G/O-NGD was compared with the effects of heparin-fixed endogenous growth factors (G/O-NGD [Heparin]). The angiogenesis effect of GFs through G/O-NGD has already been demonstrated.[23] Being a suitable carrier for GFs, it is hypothesized that G/O-NGD itself possesses distinctive properties that are expected to reduce skin damage as well as promote the healing of damaged skin along with the GFs. Hence, the significance of this study is to demonstrate and evaluate the effect of the GF-loaded G/O nanogel dispersion in the repair and prevention of UVB-induced skin damage. The G/O nanogel dispersion could be a promising carrier for transdermal delivery of GFs and, therefore, emerges a new hope in the arena of the drug delivery system.

2. MATERIALS AND METHODS

2.1. Preparation of the G/O-NGD Emulsion. Sucrose erucic acid ester (Mitsubishi Chemical Foods Co., Tokyo, Japan) was added to isopropyl myristate (Fujifilm Wako Pure Chemical Co., Osaka, Japan) and dissolved by shaking at room temperature to form the oil phase.

The ethyl(dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) solution was prepared by adding 10 mg/mL EDC (Peptide Institute Inc., Osaka, Japan) and 6 mg/mL NHS (WAKO) into a 0.05 M 2-morpholinoethanesulfonic acid monohydrate buffer (pH 5.6; Dojindo Laboratory, Kumamoto, Japan) solution. The EDC/NHS solution and heparin (10 mg/mL; WAKO) were then added to a 5% gelatin (porcine-skin-derived gelatin type A; Sigma Aldrich, St. Louis, MO) solution to form the aqueous phase. Next, the aqueous phase was mixed with the oil phase via agitation using an ultrasonic homogenizer (Sonifier 250; Branson Ultrasonic Co, Danbury, CT) at 1 pulse per 0.7 s, high-speed stirring, and 70% power amplitude resulting in a milky-form liquid. The prepared emulsion was cooled at 4 °C to jellify the aqueous phase into particles to obtain the G/O-NGD. The prepared G/O-NGD was monodispersed in nature (PdI < 0.3) with a mean particle diameter of <250 nm, as obtained in the previous study.[23]

2.2. G/O Emulsion with a Mixture of Five GFs. The five proliferation factors selected were VEGF (glycoprotein associated with vascular renewal), epithelial growth factor (EGF; associated with DNA synthesis and cell proliferation), bFGF (associated with the development of the nervous system, lungs, muscles, bone, and skin, along with vascular proliferation), TGF-1 (associated with tissue development, cell differentiation, and embryonic development), and IGF-α (associated with regeneration of damaged cells and skin). The G/O-NGD was prepared in the same manner as described in Section 2.1, except that the aqueous phase contained a mixture of the above five proliferation factors. Here, stirring was performed for 3 min with a 30 s interval to prevent the denaturation of GFs by high temperature. In this emulsion, fine nanometer-sized particles were formed and dispersed in oil. The prepared emulsion was cooled to 4 °C to allow gelation of the aqueous phase within the particles to produce the G/O emulsion.

https://doi.org/10.1021/acsomega.2c07343
ACS Omega 2023, 8, 9239−9249

2.3. In Vivo Evaluation of the Preventive and Therapeutic Effects of the G/O-NGD on UVB Radiation-Induced Damage. *2.3.1. UVB Skin Trauma Mouse Model.* Hairless nude male mice (4 weeks old, wt. 19−24 g, by Japan SLC) were fixed in cages and provided a standard diet ad libitum. All animals were treated according to the protocol approved by the Ethics Committee on Animal Experiments of Kyushu University (A21-335-1; July 9, 2021). UVB irradiation was performed only once on day 0, with the mice under anesthesia, by placing a UVB irradiator on top of the cage. UVB light was placed directly above the mouse at a distance of 8 cm, and the intensity of the irradiated UVB light was calculated as 105.6 mJ/cm^2. UVB exposure was 220.1 ± 1.7 μW/cm^2. According to the formula for light energy, UVB light energy (mJ/cm^2) = UVB intensity (mW/cm^2) × irradiation time (s). The intensity of the irradiated UVB light was calculated as 105.648 ± 0.816 mJ/cm^2. Mice were exposed to UVB radiation for 8 min and developed a damaged skin condition equivalent to a state of imbalanced skin for a month (Figure S1).

The specific experimental conditions are listed in Table 1. Group I was the no-treatment control group; group II was the prevention group: irradiation for 8 min on day 0 and untreated UVB-only irradiation, immediately followed by six days of preventive conditions for G/O-NGD application; group III was the short-term treatment group: day 0 irradiation was followed by skin damage reactions, such as visible erythema, blistering, and peeling, on day 3; a three-day short-term treatment with the application of G/O-NGD and a control without treatment; and group IV was the long-term treatment group: the conditions were set as in the three groups but with six days of applied lotion long-term treatment and no-treatment control.

Table 1. Experiment Conditions

group name	types of treatment received
group I: no-treatment group	no treatment
	UVB irradiation
group II: prevention group	UVB irradiation + G/O (Heparin)
	UVB irradiation + G/O (GF cocktail)
group III: short-term treatment group	UVB irradiation + G/O (Heparin)
	UVB irradiation + G/O (GF cocktail)
group IV: long-term treatment group	UVB irradiation
	UVB irradiation + G/O (Heparin)
	UVB irradiation + G/O (GF cocktail)

The experimental duration was determined based on significant changes in skin moisture as well as in the skin structure after UVB irradiation. GF-loaded G/O-NGD was applied to the distinctive treatment groups on days 3, 6, and 9 to determine the duration of treatment for skin repair as well as for damage prevention. Similarly, G/O-NGD application for upto 9 days reveals the safety of transdermal application of the developed nanogel dispersion for long-term use in skin. Furthermore, body weight and skin moisture tests were performed daily and transepidermal water loss tests were performed using a skin evaporative water logger (LOZENSTAR SR-101).

2.4. Histological Analysis. Skin from the backs of the mice was harvested for histological analysis on day 7 of UV exposure. This treated dorsal skin was fixed in a 10% formalin-neutral buffer solution (FUJIFILM Wako Pure Chemical Corporation). Separate paraffin-embedded blocks embedded in paraffin sections were prepared using standard techniques and visualized by hematoxylin and eosin (H&E) staining and immunohistochemical staining of macrophages with biotin-labeled anti-CD68 antibody (Abcam).

2.5. Statistical Analyses. All test results are expressed as the mean ± standard deviation. Significant differences between the two groups were analyzed using one-way analysis of variance. Statistical significance was set at $P < 0.05$ and $P < 0.001$.

3. RESULTS

3.1. Prevention of UVB Irradiation-Induced Erythema and Epidermal Dehydration by the G/O-NGD. Figure 1A

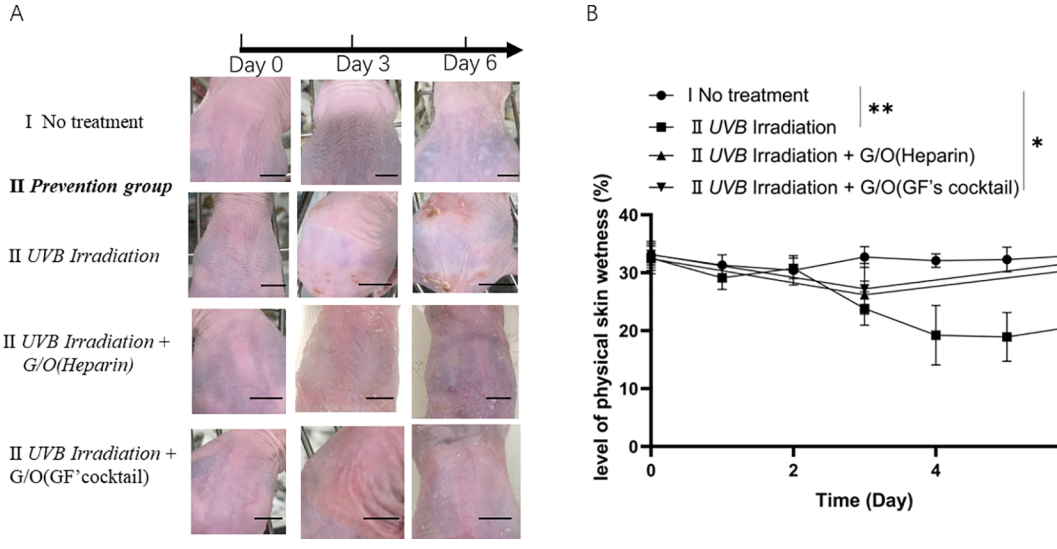


Figure 1. Inhibition of skin erythema and dehydration using the gel-in-oil nanogel dispersion (G/O-NGD) in the prevention group. (A) Images of transtemporal changes on the back skin of mice after ultraviolet (UV)-B irradiation and degree of skin damage with and without application of G/O-NGD. (B) Transcutaneous changes in skin humidity on the back of mice in the prevention group. Data are presented as the mean ± standard deviation. $n = 4$, $^*P < 0.05$, $^{**}P < 0.01$. Scale bar = 10 mm.

https://doi.org/10.1021/acsomega.2c07343
ACS Omega 2023, 8, 9239−9249

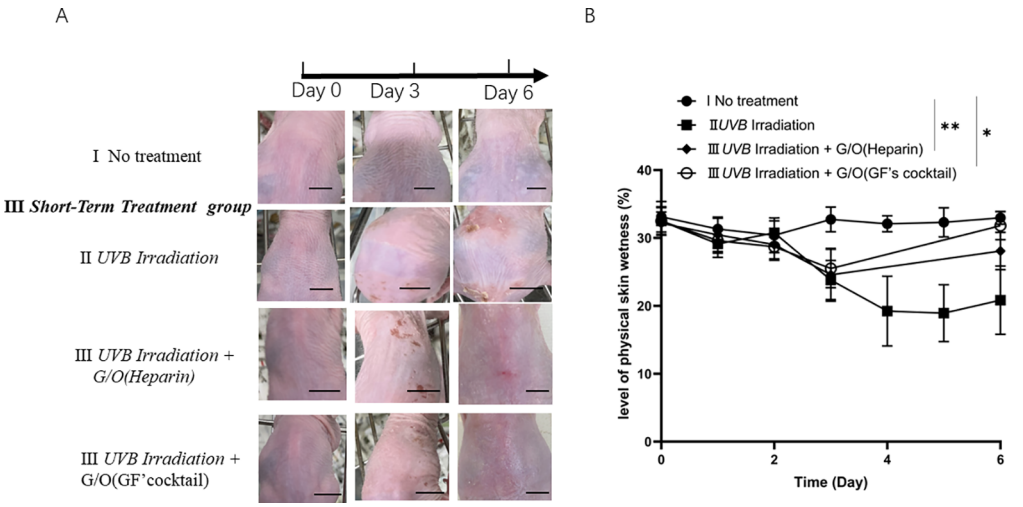


Figure 2. Inhibition of skin erythema and dehydration using G/O-NGD in the short-term treatment group. (A) Images of transtemporal changes on the back skin of mice after UVB irradiation and degree of skin damage with and without the application of G/O-NGD. (B) Transcutaneous changes in skin humidity on the back of mice in the short-term treatment group. Data are presented as the mean ± standard deviation. $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$. Scale bar = 10 mm.

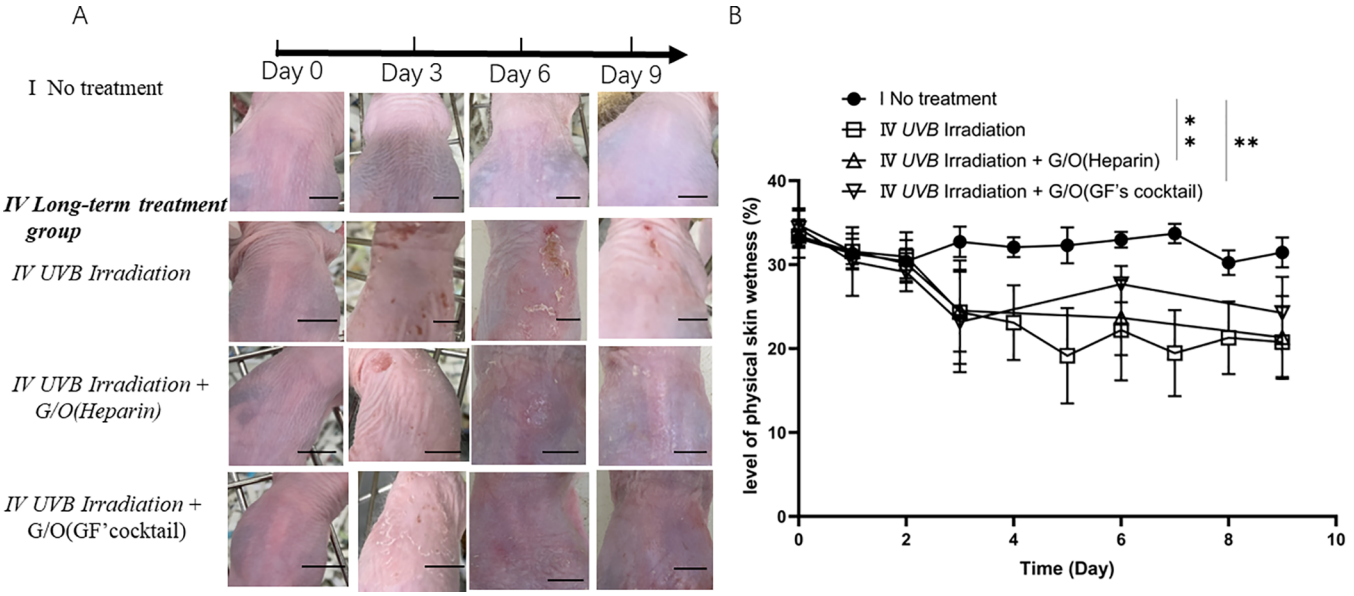


Figure 3. Inhibition of skin erythema and dehydration using G/O-NGD in the long-term treatment group. (A) Images of transtemporal changes on the back skin of mice after UVB irradiation and degree of skin damage with and without the application of G/O-NGD. (B) Transcutaneous changes in skin humidity on the back of mice in the long-term treatment group. Data are presented as the mean ± standard deviation. $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$. Scale bar = 10 mm.

shows photographs of the dorsal condition of mice in group II (prevention group) over time. Comparing the no-treatment group with the UVB irradiation (no application) condition, significant erythema was observed on the mice's skin after three days of UVB irradiation. After comparing the UVB irradiation-only, UVB irradiation + G/O (Heparin), and UVB irradiation + G/O (GF cocktail) conditions on day 3, we found that G/O application conditions prevented skin wounds. Similarly, Figure 1B shows the skin moisture level for the prevention group. The results showed that UVB radiation caused skin irritation that significantly decreased the moisture level of skin from approximately 33 to 20% over time. The skin moisture level for UVB irradiation + G/O (Heparin) and UVB irradiation + G/O (GF cocktail) on day 3 was slightly greater than the UVB radiation-only condition. However, the skin humidity in the G/O (Heparin) and G/O (GF cocktail) conditions was the same as in the ideal skin condition on day 6. Since a similar effect on the skin humidity level was achieved, it is assumed that the G/O-NGD has a preventive effect on moisture loss caused by UVB radiation-induced skin damage.

Figure 2A shows a photograph of the back of group III (short-term treatment group) mice over time. Prominent erythema on the back was observed three days after irradiation (day 3). Wound healing was expected from day 3. As both G/O (Heparin) and G/O (GF cocktail) were applied on day 3, a significant reduction in erythema was observed after three days (day 6). Figure 2B shows the skin epidermal moisture results for the third group, indicating that G/O (GF cocktail) was

https://doi.org/10.1021/acsomega.2c07343
ACS Omega 2023, 8, 9239−9249

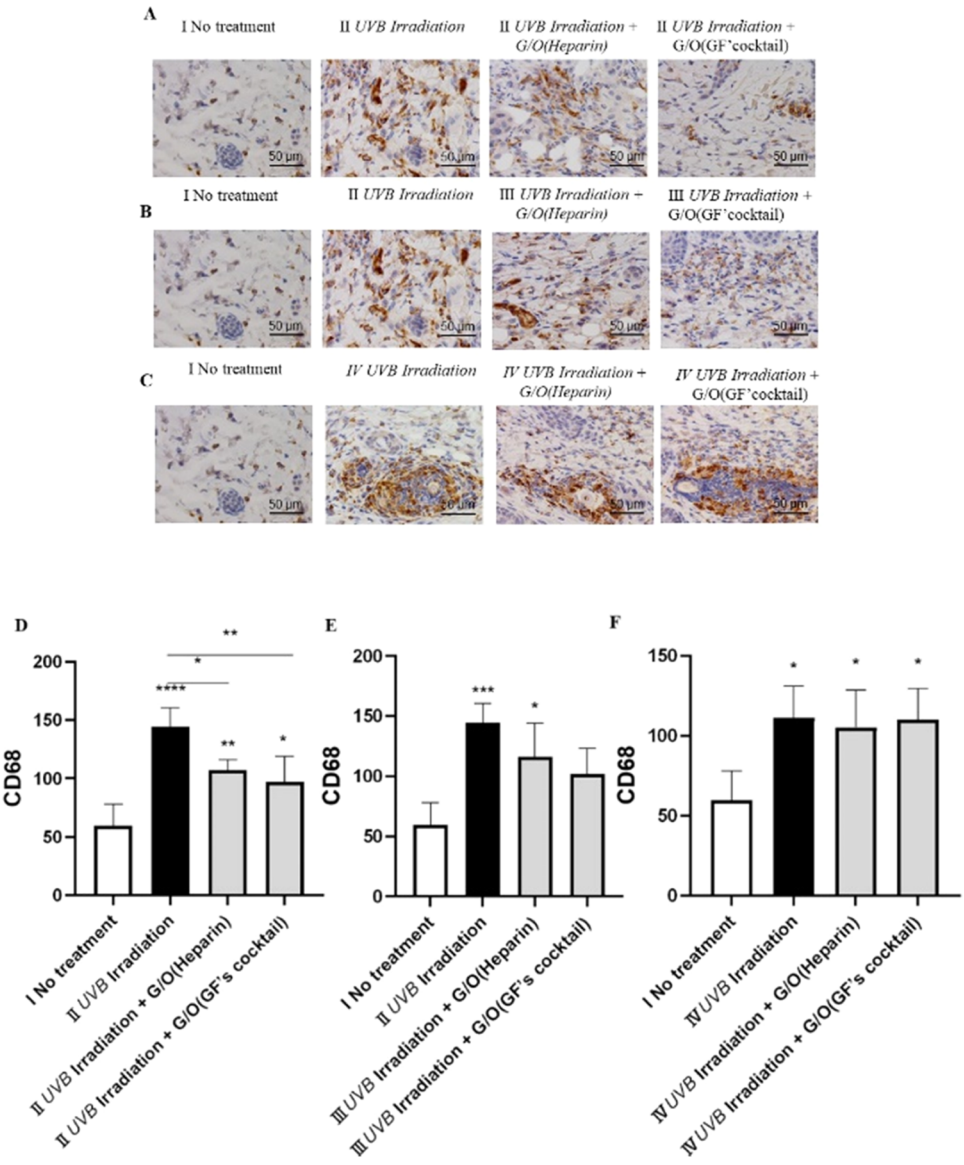


Figure 4. CD68 staining of the mouse dorsal skin tissue. (A) Prevention, (B) short-term treatment, and (C) long-term treatment groups. Macrophage counts in the (D) prevention, (E) short-term treatment, and (F) long-term treatment groups. Data are presented as the mean ± standard deviation. $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$. Scale bar = 50 μm.

more effective than G/O (Heparin) in improving the skin moisture level over a shorter treatment period.

Figure 3A shows photographs of the dorsum of mice in the fourth group (long-term treatment group) over time, comparing the no-treatment and UVB irradiation-only (no application) conditions. Significant erythema was observed three days after irradiation (day 3). Three days after G/O application (day 6), there were no visible skin wounds in the UVB irradiation + G/O (Heparin) and UVB irradiation + G/O (GF cocktail) conditions, and photographs of pure UVB irradiation from days 6 to 9 showed that the wound sites recovered due to the spontaneous healing effects on the mice, but healing was faster in the case of G/O application. Figure 3B shows the skin moisture results in group IV. The lower skin humidity in the UVB irradiation-only condition compared to the no-treatment condition confirms the roughness of the skin caused by UVB irradiation and other conditions. The higher skin humidity of G/O (Heparin) compared to G/O (GF cocktail) conditions suggests that G/O (GF cocktail) treatment is more effective.

3.2. Immunohistochemistry of the Skin. Figure 4 shows a graph of the microscopic observations of the skin after CD68 staining and the quantitative results of macrophage counts. CD68 staining of the prevention group and quantification of macrophages (Figure 4A,D) revealed a significant increase in macrophage markers under UVB irradiation-only conditions, indicating macrophage accumulation at the wound site. In contrast, under G/O (Heparin) and G/O (GF cocktail) conditions, macrophage expression was lower than that under UVB irradiation only, indicating that G/O has a preventive effect on inflammation at the wound site. The results of CD68 staining from the short-term treatment group (Figure 4B,E) showed that the macrophage markers were lowest in G/O (GF cocktail), indicating that G/O (GF cocktail) treatment has good control on the inflammation that is better than that of G/O (Heparin) treatment. From the results of the long-term treatment group (Figure 4C,F), the number of macrophages

https://doi.org/10.1021/acsomega.2c07343
ACS Omega 2023, 8, 9239−9249

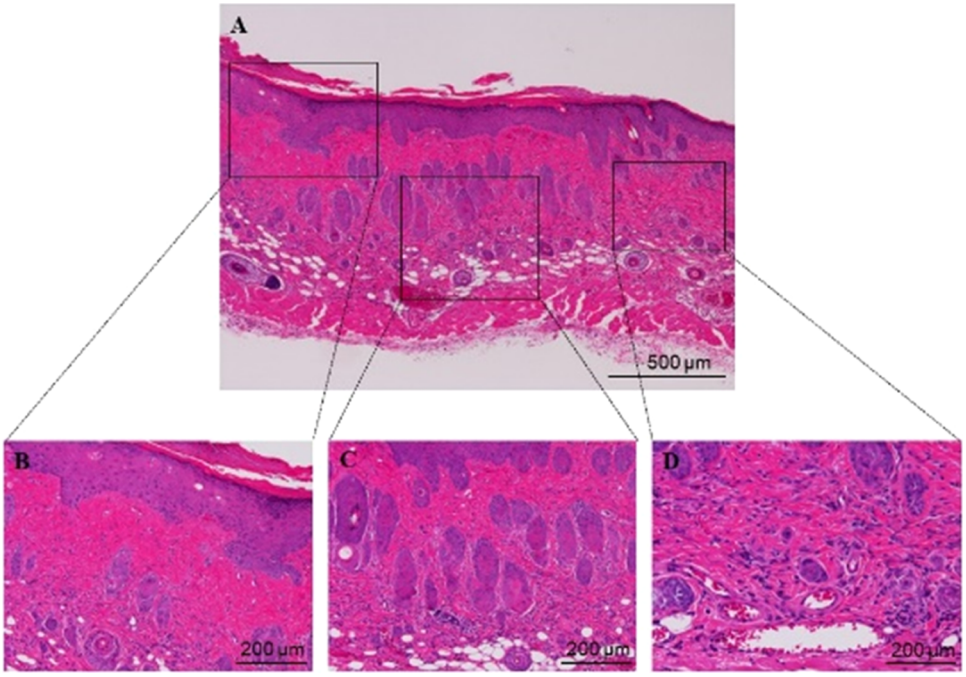


Figure 5. Histological analysis of the mice skin in the no-treatment group. (A) Hematoxylin and eosin (H&E)-stained images of the dorsal skin of mice. (B) Skin tissue fibrosis. (C) Increased number of hair follicles. (D) Inflammation. Scale bar: A = 500 μm; B, C, and D = 200 μm.

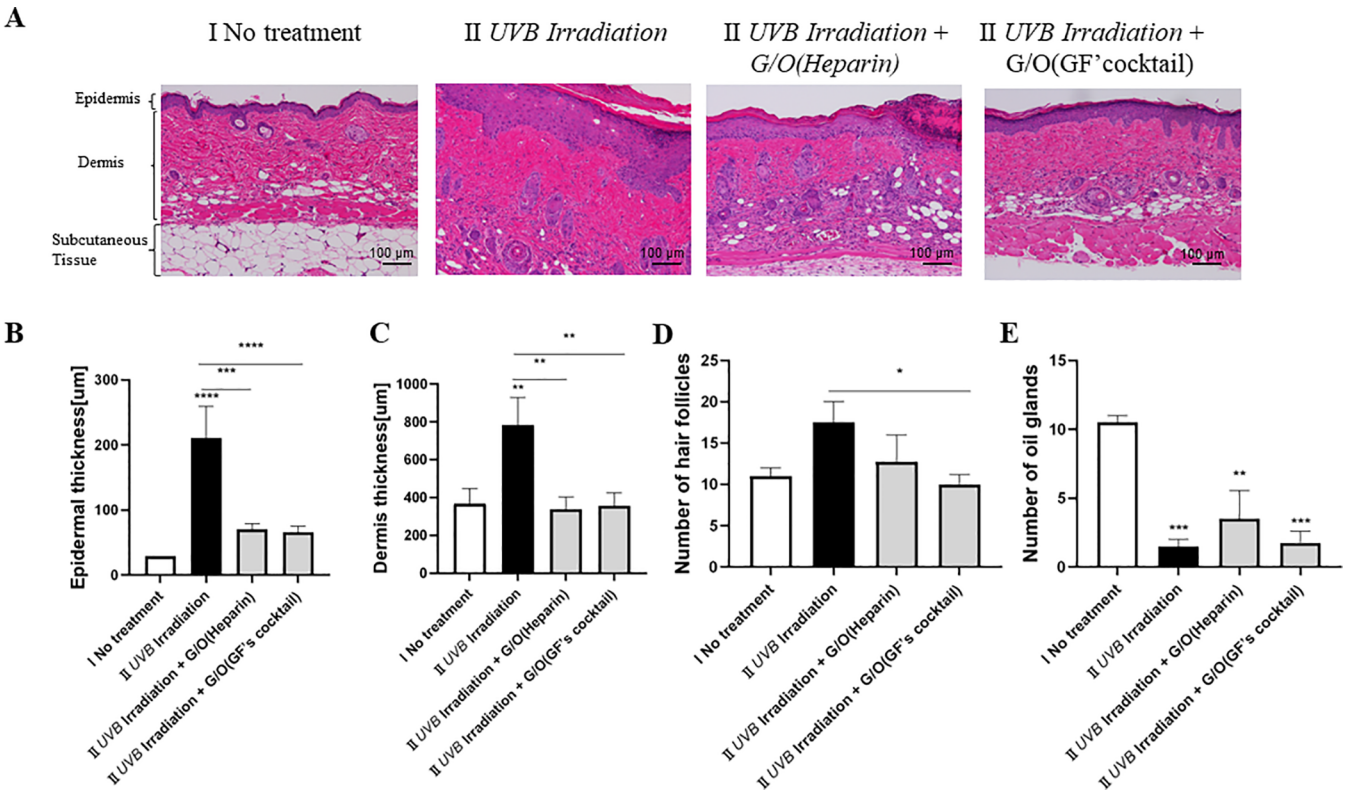


Figure 6. Histological analysis of the mice skin in the prevention group. (A) H&E-stained images of the dorsal skin of mice. (B) Epidermal thickness. (C) Dermis thickness. (D) Number of hair follicles. (E) Number of sebaceous glands. Data are presented as the mean ± standard deviation. n = 4, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Scale bar = 100 μm.

was the same for UVB irradiation, G/O (Heparin), and G/O (GF cocktail) conditions. Comparing the results of the short-term treatment groups, we believe that the reduction in the number of macrophages in the irradiation-only group was caused by spontaneous healing in mice.

3.3. Histological Analysis. On the 7th day after UVB irradiation, histological analysis of the mouse back skin was performed via H&E staining. H&E-stained images of the dorsal skin of mice (Figure 5A) were observed for possible skin lesions induced by UVB radiation in group I. Various skin

https://doi.org/10.1021/acsomega.2c07343
ACS Omega 2023, 8, 9239−9249

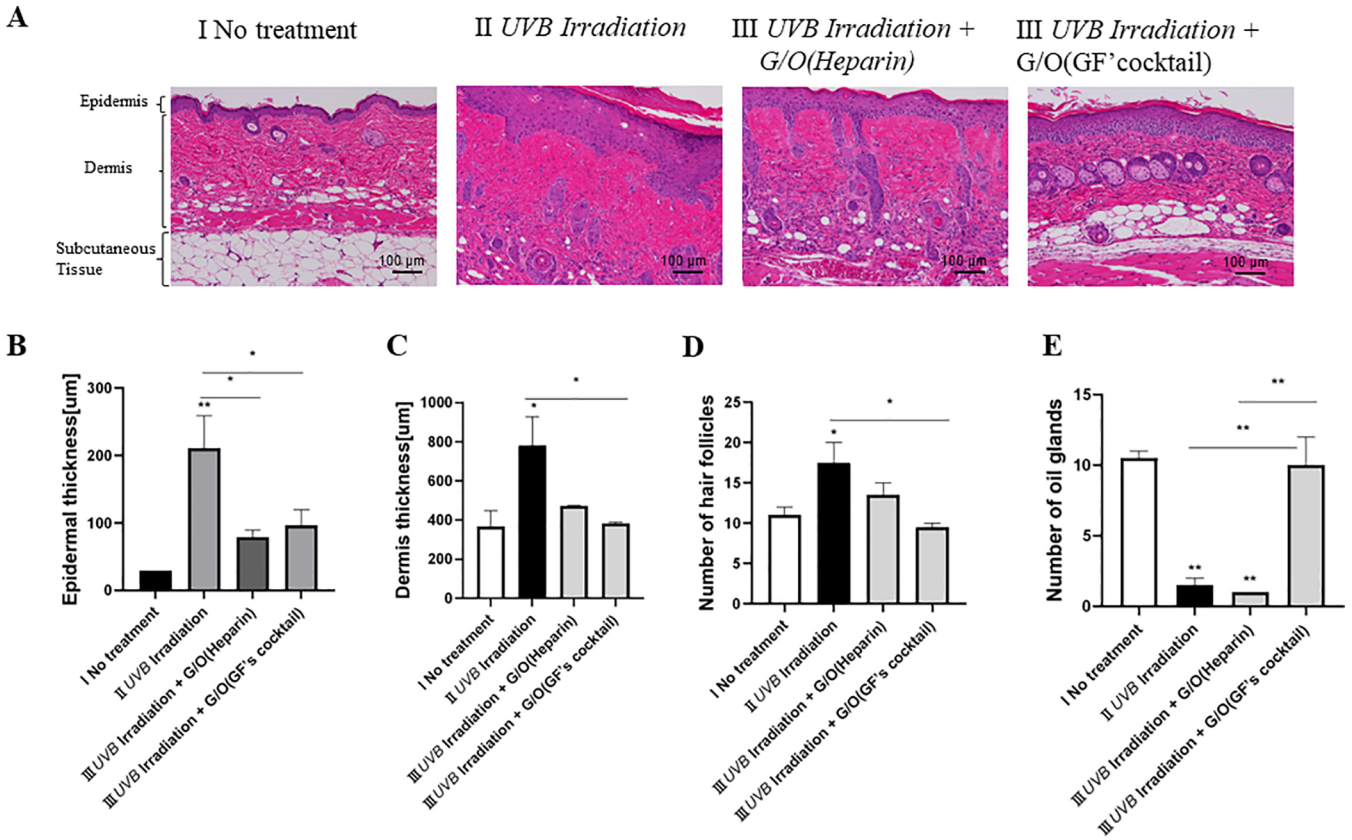


Figure 7. Histological analysis of the mice skin in the short-term treatment group. (A) H&E-stained images of the dorsal skin of mice. (B) Epidermal thickness. (C) Dermis thickness. (D) Number of hair follicles. (E) Number of sebaceous glands. Data are presented as the mean ± standard deviation. $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Scale bar = 100 μm.

lesions, such as marked epidermal and dermal hypertrophy, skin tissue fibrosis (Figure 5B), increased number of hair follicles (Figure 5C), and inflammation (Figure 5D), were observed during the histological analysis.

H&E staining results for the prevention group (Figure 6A) revealed that G/O (Heparin) and G/O (GF cocktail) were effective in preventing epidermal and dermal thickening induced by UVB irradiation (Figure 6B,C). Furthermore, a significant increase in the number of hair follicles was observed in the UVB irradiation-only group (Figure 6D), indicating that G/O administration suppressed the abnormal increase in the number of hair follicles. Under UVB irradiation, the number of sebaceous glands was reduced with or without the application of the G/O-NGD (Figure 6E).

Histological analysis of the short-term treatment group after H&E staining (Figure 7A) showed that the application of G/O (Heparin) and G/O (GF cocktail) was effective in ameliorating epidermal and dermal thickening induced by UVB irradiation (Figure 7B,C). The results of hair follicle counts showed that UVB irradiation alone caused a significant increase, whereas the application of G/O suppressed the increase in hair follicle counts (Figure 7D). The results for sebaceous glands (Figure 7E) revealed that the G/O (GF cocktail) condition significantly restored the number of oil-producing glands in the skin.

The results for the long-term treatment group (Figure 8A) revealed that the G/O-NGD treatment improved epidermal thickening (Figure 8B). Meanwhile, there was no significant difference in dermal thickness in the absence or presence of G/O-NGD, G/O (Heparin), and G/O (GF cocktail) (Figure 8C), possibly due to the spontaneous healing effect in mice.

The results for the follicle number showed that UVB irradiation alone caused a significant increase in the hair follicle number, but G/O application inhibited this effect (Figure 8D). Moreover, the G/O (GF cocktail) condition did not have any effect on repairing the oil glands (Figure 8E); however, as there was no significant difference, we speculate this to be caused by an experimental error.

4. DISCUSSION

Previously, we developed a G/O emulsion for the transdermal delivery of biomolecules. This G/O emulsion was validated as a suitable drug delivery vehicle with an optimal particle size to easily penetrate the skin and cause slow drug release into the dermis of the skin.[23] In this study, we investigated the role of G/O emulsions in the healing of skin damage. Using a UVB-induced skin injury model in hairless mice, we investigated whether the application of the G/O-NGD emulsion encapsulated in a cocktail of five GFs affected the UVB-induced skin sunburn. Transdermal application of the G/O-NGD accelerated the inhibition of UVB-induced skin damage, such as erythema, reduced skin epidermal moisture, inflammation, and epidermal thickening caused by sunburn. Additionally, G/O-NGD inhibited UVB-induced abnormal hair follicle growth. G/O-NGD, which encapsulated five GFs, also demonstrated rapid short-term repair of damaged sebaceous glands. Hence, this study provides a new approach to the treatment of UVB-induced skin damage.

https://doi.org/10.1021/acsomega.2c07343
ACS Omega 2023, 8, 9239−9249

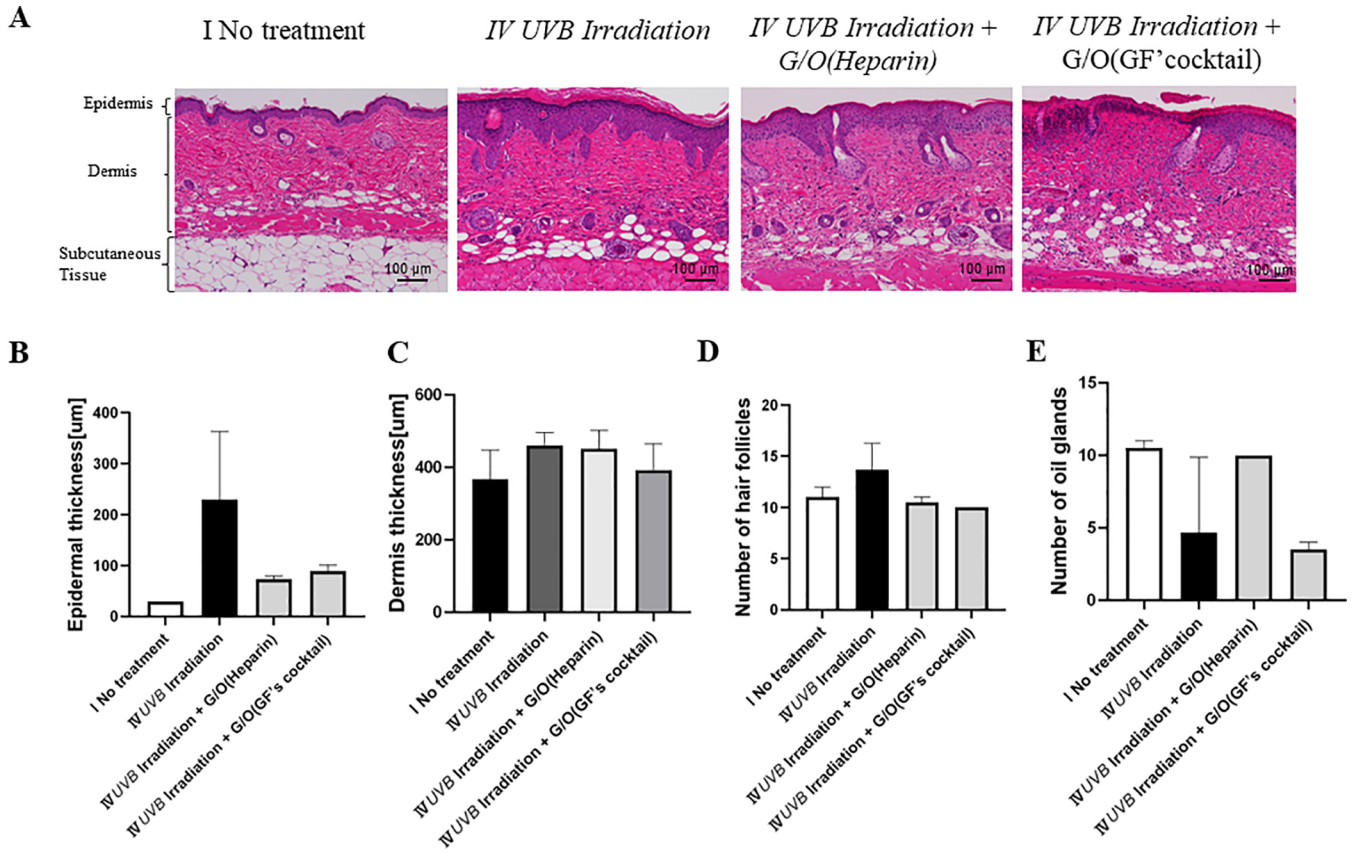


Figure 8. Histological analysis of the mice skin in the long-term treatment group. (A) H&E-stained images of the dorsal skin of mice. (B) Epidermal thickness. (C) Dermis thickness. (D) Number of hair follicles. (E) Number of sebaceous glands. Data are presented as the mean ± standard deviation. $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Scale bar = 100 μm.

The results of this study on the degree of skin damage and change in epidermal water content in the prevention (Figure 1), short-term treatment (Figure 2), and long-term treatment (Figure 3) groups indicate that the application of G/O-NGD restored the moisturizing effect of the skin. There are three possible reasons for this finding: first, modern wound healing theory confirms that a moist environment prevents wound dehydration and favors wound healing by increasing collagen synthesis and angiogenesis, along with an increased breakdown of dead cells, and requires oxygen.[26−29] G/O-NGD is an oil-in-nanogel emulsion, which provides a hermetic barrier for wound treatment, prevents contamination, and allows moist wound healing. The soft texture of the nanohydrogel also provides comfort during treatment. Another reason is the moisturizing effect of heparin. Heparin is a polysaccharide that can adsorb water and is suggested to have highly moisturizing properties.[30,31] Heparin also has good analgesic and anti-inflammatory effects, and its moisturizing effect can restore skin moisture.[32] A third possible reason is the effect of GFs' on wound healing. The five GFs used in this study contribute to skin regeneration, which may restore skin moisture.[33−36]

Although there was a slight reduction in skin water loss in the prevention group, the difference was not statistically significant. These findings suggest that the restoration of skin moisture did not occur suddenly or immediately after the use of G/O-NGD but gradually over time. As the recovery of skin moisture in this experiment was not rapid after G/O-NGD application, we believe that the recovery of skin moisture was more likely due to the secondary effect of GFs on wound healing rather than the direct moisturizing effect of heparin. However, the comparison between G/O (Heparin) and G/O (GF cocktail) conditions showed a slight difference in skin moisture levels. This may be due to the greater effect of endogenous GFs bound to heparin compared to exogenous GFs. Heparin can capture GFs and is effective in treating trauma.[24] Therefore, endogenous cellular GFs may be activated by heparin, and superior wound healing can be achieved via endogenous GF anchoring in heparin. Nevertheless, we do not believe that the introduction of external GFs is meaningless because the skin humidity was higher in the G/O (GF cocktail) condition than in the G/O (Heparin) condition, although the difference was not significant. These observations are only based on appearance and skin humidity.

Skin damage caused by UVB irradiation is multifaceted and is not only related to visible wounds on the skin surface and changes in the moisture content of the epidermis but also to changes in epidermal hyperkeratosis, thickening of the stroma, dermal vasodilation, and the number of hair follicles.[37] We evaluated the macrophage aggregation status within the skin using CD68 staining (Figure 4). Inactivated macrophages are called M0 macrophages, and their characteristic surface markers include CD14, CD68, and F4/80.[38] After the initial stimulation, macrophages acquire different phenotypes, M1 and M2. M1 macrophages are "classically activated" with CD80 and CD86 as characteristic surface markers and are mainly involved in the inflammatory response, which is not conducive to tissue regeneration. Conversely, M2 macrophages are called "alternatively activated macrophages," with CD163

https://doi.org/10.1021/acsomega.2c07343
ACS Omega 2023, 8, 9239−9249

and CD206 as characteristic surface markers. They can suppress inflammation, participate in tissue regeneration by driving immune regulation, and promote wound healing and tissue remodeling.[39,40] Our results showed that G/O (Heparin) and G/O (GF cocktail) treatments inhibited UVB-induced macrophage expression in both the prophylactic and treatment groups. A comparison of G/O (Heparin) and G/O (GF cocktail) treatments showed a slight but not significant difference. This may be due to the CD68 staining of macrophages, including preactivation M0 and post-activation M1 and M2 macrophages. If the number of M2 macrophages was higher in the G/O (GF cocktail) treatment, the overall CD68 expression level would converge with that in the G/O (Heparin) treatment.

UVB-induced skin damage increased the thicknesses of the epidermis and dermis in the prevention (Figure 6B,C), short-term treatment (Figure 7B,C), and long-term treatment (Figure 8B,C) groups. Cell GFs can promote the healing of deep wounds that penetrate the dermis. For example, alkaline GFs achieve skin barrier repair by promoting the proliferation of dermal keratin-forming cells.[41,42] In our study, when G/O (GF cocktail) was applied to the skin surface, G/O delivered GFs to the interior of the skin to promote skin regeneration and inhibit the secretion of proinflammatory and proproliferative factors in keratin-forming cells. Therefore, a significant decrease in epidermal and dermal thickness was observed in the G/O (Heparin) and G/O (GF cocktail) administration conditions compared to the irradiated group. This leads to an inhibitory effect on UVB-induced photodamage.

Some studies on UVB damage to hair follicles have shown that UV rays upregulate the expression of the wingless/integrated (Wnt) signaling pathway, where the signaling molecule Wnt10b promotes the growth of hair follicles.[43] UVB light significantly prolongs the hair follicle cycle, which can be considered a self-protective stress response in vivo.[44] In our study, since G/O inhibited and treated the damage caused by UVB, the expression of the Wnt signaling pathway was reduced, while the number of hair follicles remained normal. As a result, the significant upregulation that occurs in the hair follicle cycle after UVB irradiation can be avoided. Therefore, the number of hair follicles remained almost similar to that in the untreated group and was much lower than that in the UVB-irradiated group after G/O (Heparin) and G/O (GF cocktail) administration in the prevention (Figure 6D), short-term treatment (Figure 7D), and long-term treatment (Figure 8D) groups.

The scaling of sebaceous glands is caused by the intercellular interactions between epidermal keratinocytes and fibroblasts; therefore, the repair of epidermal keratinized cells and fibroblasts is the key to sebaceous gland repair.[45] In the prevention group, we found that the sebaceous glands were reduced in the presence or absence of G/O-NGD (Figure 6E). In the short-term treatment group (Figure 7E), the G/O (GF cocktail) condition significantly repaired the number of sebaceous glands. Conversely, the G/O (GF cocktail) condition did not affect the sebaceous gland repair (Figure 8E) in the long-term treatment, but since no significant difference was observed, we considered this to be caused by an experimental error.

5. CONCLUSIONS

The experiments described in this work revealed that UVB-induced skin damage, such as erythema, skin moisture reduction, inflammation, and epidermal dermis thickening, can be reduced by the administration of a G/O-NGD. The G/O-NGD can also prevent and treat abnormal follicle proliferation caused by UVB-induced damage to the hair follicles. Moreover, the administration of G/O-NGD also showed potential to repair the lipid glands. Therefore, the current study proposes a new subcutaneous grafting technique via the transdermal delivery of GF-functionalized G/O nanogels, which can contribute significantly to the fields of trauma treatment and regenerative medicine.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c07343.

Construction of the UVB skin damage model (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Hiroyuki Ijima − *Department of Chemical Engineering, Faculty of Engineering, Graduate School, Kyushu University, Fukuoka 819-0395, Japan*; orcid.org/0000-0002-4737-5454; Phone: +81-92-802-2748; Email: ijima@chem-eng.kyushu-u.ac.jp

Authors

Yi Zhang − *Department of Chemical Engineering, Faculty of Engineering, Graduate School, Kyushu University, Fukuoka 819-0395, Japan*

Yuuta Inoue − *Department of Chemical Engineering, Faculty of Engineering, Graduate School, Kyushu University, Fukuoka 819-0395, Japan*

Jannatul Fardous − *Department of Pharmacy, Faculty of Science, Comilla University, Cumilla 3506, Bangladesh*

Ryota Doi − *Department of Chemical Engineering, Faculty of Engineering, Graduate School, Kyushu University, Fukuoka 819-0395, Japan*

Takahiro Ijima − *Department of Quantum Science and Energy Engineering, Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan*

Toshioh Fujibuchi − *Department of Health Sciences, Faculty of Medical Sciences, Graduate School, Kyushu University, Fukuoka 812-8582, Japan*

Yo-ichi Yamashita − *Aso Iizuka Hospital, Fukuoka 820-8505, Japan*

Shinichi Aishima − *Department of Pathology & Microbiology, Faculty of Medicine, Saga University, Saga 849-8501, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c07343

Funding

This study was partially supported by the Japan Society for the Promotion of Science (JSPS KAKENHI; grant number JP21H01732), the KOSÉ Cosmetology Research Foundation, and Nippon 363 Shokubai CO. LTD. (Japan).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to thank the members of the laboratories of Biomaterial & Medical Engineering (Ijima Lab.) for their continued support and feedback during the evolution of our

work. We would also like to thank the KOSÉ Cosmetology Research Foundation and Nippon 363 Shokubai CO. LTD. (Japan) for their continued love and support.

REFERENCES

(1) Malcov-Brog, H.; Alpert, A.; Golan, T.; Parikh, S.; Nordlinger, A.; Netti, F.; Sheinboim, D.; Dror, I.; Thomas, L.; Cosson, C.; Gonen, P.; Stanevsky, Y.; Brenner, R.; Perluk, T.; Frand, J.; Elgavish, S.; Nevo, Y.; Rahat, D.; Tabach, Y.; Khaled, M.; Shen-Orr, S. S.; Levy, C. UV-Protection Timer Controls Linkage between Stress and Pigmentation Skin Protection Systems. *Mol. Cell* **2018**, *72*, 444−456.e7.

(2) Zou, W.; González, A.; Jampaiah, D.; Ramanathan, R.; Taha, M.; Walia, S.; Sriram, S.; Bhaskaran, M.; Dominguez-Vera, J. M.; Bansal, V. Skin color-specific and spectrally-selective naked-eye dosimetry of UVA, B and C radiations. *Nat. Commun.* **2018**, *9*, No. 3743.

(3) Peakman, T.; Page, M.; Gewert, D. Increased recombinational efficiency in insect cells irradiated with short wavelength ultra-violet light. *Nucleic Acids Res.* **1989**, *17*, 5403.

(4) Montagner, A.; Delgado, M. B.; Tallichet-Blanc, C.; Chan, J. S. K.; Sng, M. K.; Mottaz, H.; Degueurce, G.; Lippi, Y.; Moret, C.; Baruchet, M.; Antsiferova, M.; Werner, S.; Hohl, D.; Saati, T. A.; Farmer, P. J.; Tan, N. S.; Michalik, L.; Wahli, W. Src is activated by the nuclear receptor peroxisome proliferator-activated receptor β/δ in ultraviolet radiation-induced skin cancer. *EMBO Mol. Med.* **2014**, *6*, 80−98.

(5) Qiang, L.; Sample, A.; Shea, C. R.; Soltani, K.; Macleod, K. F.; He, Y.-Y. Autophagy gene ATG7 regulates ultraviolet radiation-induced inflammation and skin tumorigenesis. *Autophagy* **2017**, *13*, 2086−2103.

(6) Heo, S. Y.; Kim, J.; Gutruf, P.; Banks, A.; Wei, P.; Pielak, R.; Balooch, G.; Shi, Y.; Araki, H.; Rollo, D.; Gaede, C.; Patel, M.; Kwak, J. W.; Peña-Alcántara, A. E.; Lee, K.-T.; Yun, Y.; Robinson, J. K.; Xu, S.; Rogers, J. A. Wireless, battery-free, flexible, miniaturized dosimeters monitor exposure to solar radiation and to light for phototherapy. *Sci. Transl. Med.* **2018**, *10*, No. eaau1643.

(7) Reveguk, Z. V.; Khoroshilov, E. V.; Sharkov, A. V.; Pomogaev, V. A.; Buglak, A. A.; Tarnovsky, A. N.; Kononov, A. I. Exciton Absorption and Luminescence in i-Motif DNA. *Sci. Rep.* **2019**, *9*, No. 15988.

(8) Prausnitz, M. R.; Langer, R. Transdermal drug delivery. *Nat. Biotechnol.* **2008**, *26*, 1261−1268.

(9) He, X.; Louis, N.; Katagi, H.; Zhang, P.; Balyasnikova, I.; Horbinski, C.; Goldman, S.; Hashizume, R. Dipg-33. New Therapeutic Approach for Brainstem Glioma: Intranasal Delivery of Nanoliposomal Sn-38. *Neuro Oncol.* **2018**, *20*, i55.

(10) Yao, X.; Chen, D.; Zhao, B.; Yang, B.; Jin, Z.; Fan, M.; Tao, G.; Qin, S.; Yang, W.; He, Q. Acid-Degradable Hydrogen-Generating Metal-Organic Framework for Overcoming Cancer Resistance/Metastasis and Off-Target Side Effects. *Adv. Sci.* **2022**, *9*, No. e2101965.

(11) Wei, X.; Shao, B.; He, Z.; Ye, T.; Luo, M.; Sang, Y.; Liang, X.; Wang, W.; Luo, S.; Yang, S.; Zhang, S.; Gong, C.; Gou, M.; Deng, H.; Zhao, Y.; Yang, H.; Deng, S.; Zhao, C.; Yang, L.; Qian, Z.; Li, J.; Sun, X.; Han, J.; Jiang, C.; Wu, M.; Zhang, Z. Cationic nanocarriers induce cell necrosis through impairment of Na(+)/K(+)-ATPase and cause subsequent inflammatory response. *Cell Res.* **2015**, *25*, 237−253.

(12) Mi, Y.; Hagan, C. T. t.; Vincent, B. G.; Wang, A. Z. Emerging Nano-/Microapproaches for Cancer Immunotherapy. *Adv. Sci.* **2019**, *6*, No. 1801847.

(13) Yin, Q.; Pan, A.; Chen, B.; Wang, Z.; Tang, M.; Yan, Y.; Wang, Y.; Xia, H.; Chen, W.; Du, H.; Chen, M.; Fu, C.; Wang, Y.; Yuan, X.; Lu, Z.; Zhang, Q.; Wang, Y. Quantitative imaging of intracellular nanoparticle exposure enables prediction of nanotherapeutic efficacy. *Nat. Commun.* **2021**, *12*, No. 2385.

(14) Zhao, X.; Liu, Y.; Shao, C.; Nie, M.; Huang, Q.; Li, J.; Sun, L.; Zhao, Y. Photoresponsive Delivery Microcarriers for Tissue Defects Repair. *Adv. Sci.* **2019**, *6*, No. 1901280.

(15) Bikfalvi, A.; Klein, S.; Pintucci, G.; Rifkin, D. B. Biological roles of fibroblast growth factor-2. *Endocr. Rev.* **1997**, *18*, 26−45.

(16) Wong, W. T.; Cooke, J. P. Therapeutic transdifferentiation of human fibroblasts into endothelial cells using forced expression of lineage-specific transcription factors. *J. Tissue Eng.* **2016**, *7*, No. 2041731416628329.

(17) Xuan, Y. H.; Huang, B. B.; Tian, H. S.; Chi, L. S.; Duan, Y. M.; Wang, X.; Zhu, Z. X.; Cai, W. H.; Zhu, Y. T.; Wei, T. M.; Ye, H. B.; Cong, W. T.; Jin, L. T. High-glucose inhibits human fibroblast cell migration in wound healing via repression of bFGF-regulating JNK phosphorylation. *PLoS One* **2014**, *9*, No. e108182.

(18) Yang, Y.; Kim, H. J.; Woo, K. J.; Cho, D.; Bang, S. I. Lipo-PGE1 suppresses collagen production in human dermal fibroblasts via the ERK/Ets-1 signaling pathway. *PLoS One* **2017**, *12*, No. e0179614.

(19) Kim, Y. W.; Baek, S. R.; Lee, E. S.; Lee, S. H.; Moh, S. H.; Kim, S. Y.; Moh, J. H.; Kondo, C.; Cheon, Y. W. Wound healing effects of rose placenta in a mouse model of full-thickness wounds. *Arch. Plast. Surg.* **2015**, *42*, 686−694.

(20) Hou, Y.-T.; Ijima, H.; Shirakigawa, N.; Takei, T.; Kawakami, K. Development of growth factor-immobilizable material for hepatocyte transplantation. *Biochem. Eng. J.* **2012**, *69*, 172−181.

(21) Zhou, C.; Guo, X.; Wang, S.; Zhu, Y.; Mu, D. Effects of temperature and additives on stability and spectrum of a therapeutic fibroblast growth factor. *Daru* **2011**, *19*, 138−144.

(22) Ikegami, Y.; Mizumachi, H.; Yoshida, K.; Ijima, H. Heparin-conjugated collagen as a potent growth factor-localizing and stabilizing scaffold for regenerative medicine. *Regener. Ther.* **2020**, *15*, 236−242.

(23) Fardous, J.; Yamamoto, E.; Omoso, Y.; Nagao, S.; Inoue, Y.; Yoshida, K.; Ikegami, Y.; Zhang, Y.; Shirakigawa, N.; Ono, F.; Ijima, H. Development of a gel-in-oil emulsion as a transdermal drug delivery system for successful delivery of growth factors. *J. Biosci. Bioeng.* **2021**, *132*, 95−101.

(24) Joshi, A.; Xu, Z.; Ikegami, Y.; Yoshida, K.; Sakai, Y.; Joshi, A.; Kaur, T.; Nakao, Y.; Yamashita, Y.-i.; Baba, H.; Aishima, S.; Singh, N.; Ijima, H. Exploiting synergistic effect of externally loaded bFGF and endogenous growth factors for accelerated wound healing using heparin functionalized PCL/gelatin co-spun nanofibrous patches. *Chem. Eng. J.* **2021**, *404*, No. 126518.

(25) de Gruijl, F. R.; van Kranen, H. J.; Mullenders, L. H. UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. *J. Photochem. Photobiol., B* **2001**, *63*, 19−27.

(26) żywicka, A.; Peitler, D.; Rakoczy, R.; Junka, A. F.; Fijałkowski, K. Wet and dry forms of bacterial cellulose synthetized by different strains of Gluconacetobacter xylinus as carriers for yeast immobilization. *Appl. Biochem. Biotechnol.* **2016**, *180*, 805−816.

(27) Sood, A.; Granick, M. S.; Tomaselli, N. L. Wound Dressings and Comparative Effectiveness Data. *Adv. Wound Care* **2014**, *3*, 511−529.

(28) Purnamawati, S.; Indrastuti, N.; Danarti, R.; Saefudin, T. The role of moisturizers in addressing various kinds of dermatitis: a review. *Clin. Med. Res.* **2017**, *15*, 75−87.

(29) Junker, J. P.; Kamel, R. A.; Caterson, E. J.; Eriksson, E. Clinical Impact Upon Wound Healing and Inflammation in Moist, Wet, and Dry Environments. *Adv. Wound Care* **2013**, *2*, 348−356.

(30) Hamza, F. A.; Salim, A. A.-E.; Mohammed, H. N. R. Evaluation of the Effect of Topical Heparin on the Treatment of Facial Burn. *Egypt. J. Hosp. Med.* **2019**, *77*, 5455−5460.

(31) Labetoulle, M.; Rousseau, A.; M'Garrech, M.; Dupas, B.; Baudouin, C.; Barreau, E.; Bourcier, T.; Chiambaretta, F. Efficacy of heparin sulfate mimetic polymer in Cogan's epithelial dystrophy. *Invest. Ophthalmol. Visual Sci.* **2017**, *58*, 2633.

(32) Imokawa, G. Ceramides as Natural Moisturizing Factors. In *Skin Moisturization*; Informa, 2002; p 267.

(33) Markowska, A. I.; Liu, F. T.; Panjwani, N. Galectin-3 is an important mediator of VEGF- and bFGF-mediated angiogenic response. *J. Exp. Med.* **2010**, *207*, 1981−1993.

https://doi.org/10.1021/acsomega.2c07343
ACS Omega 2023, 8, 9239−9249

(34) Lou, Z.; Lou, Z.; Jiang, Y.; Chen, Z. FGF2 and EGF for the Regeneration of Tympanic Membrane: A Systematic Review. *Stem Cells Int.* **2021**, *2021*, No. 2366291.
(35) Zhou, T.; Li, X.; Li, G.; Tian, T.; Lin, S.; Shi, S.; Liao, J.; Cai, X.; Lin, Y. Injectable and thermosensitive TGF-β1-loaded PCEC hydrogel system for in vivo cartilage repair. *Sci. Rep.* **2017**, *7*, No. 10553.
(36) Hsu, C. F.; Huang, H. S.; Chen, P. C.; Ding, D. C.; Chu, T. Y. IGF-axis confers transformation and regeneration of fallopian tube fimbria epithelium upon ovulation. *EBioMedicine* **2019**, *41*, 597−609.
(37) Oh, C. T.; Lee, D.; Koo, K.; Lee, J.; Yoon, H. S.; Choi, Y. M.; Kwon, T.-R.; Kim, B. J. Superoxide dismutase 1 inhibits alpha-melanocyte stimulating hormone and ultraviolet B-induced melanogenesis in murine skin. *Ann. Dermatol.* **2014**, *26*, 681−687.
(38) Krzyszczyk, P.; Schloss, R.; Palmer, A.; Berthiaume, F. The Role of Macrophages in Acute and Chronic Wound Healing and Interventions to Promote Pro-wound Healing Phenotypes. *Front. Physiol.* **2018**, *9*, No. 419.
(39) Castoldi, A.; de Souza, N.; Câmara, C.; Moraes-Vieira, N. O. The Macrophage Switch in Obesity Development. *Front. Immunol.* **2015**, *6*, No. 637.
(40) Atri, C.; Guerfali, F. Z.; Laouini, D. Role of Human Macrophage Polarization in Inflammation during Infectious Diseases. *Int. J. Mol. Sci.* **2018**, *19*, No. 1801.
(41) Piipponen, M.; Li, D.; Landén, N. X. The Immune Functions of Keratinocytes in Skin Wound Healing. *Int. J. Mol. Sci.* **2020**, *21*, No. 8790.
(42) Berry-Kilgour, C.; Cabral, J.; Wise, L.; et al. Advancements in the Delivery of Growth Factors and Cytokines for the Treatment of Cutaneous Wound Indications. *Adv. Wound Care* **2021**, *10*, 596−622.
(43) Wu, Z.; Zhu, Y.; Liu, H.; Liu, G.; Li, F. Wnt10b promotes hair follicles growth and dermal papilla cells proliferation via Wnt/β-Catenin signaling pathway in Rex rabbits. *Biosci. Rep.* **2020**, *40*, No. BSR20191248.
(44) Zhai, X.; Gong, M.; Peng, Y.; Yang, D. Effects of UV induced-photoaging on the hair follicle cycle of C57BL6/J mice. *Clin., Cosmet. Invest. Dermatol.* **2021**, *14*, 527.
(45) Ojima, S.; Akimoto, N.; Sugimoto, H.; Tanaka, S.; Hirayama, M.; Takenaka, Y.; Tsunemi, Y.; Kawashima, M.; Sato, T. The distinct effect of UVB and near-infrared radiation on sebum secretion in differentiated hamster sebocytes. *J. Dermatol. Sci.* **2016**, *84*, No. e170.

https://doi.org/10.1021/acsomega.2c07343
ACS Omega 2023, 8, 9239−9249