Check for updates

Skin Research and Technology

WILEY

ORIGINAL ARTICLE OPEN ACCESS

The Effect of Polynucleotide-Hyaluronic Acid Hydrogel in the Recovery After Mechanical Skin Barrier Disruption

Ye Jin Ha[1] | Ka Hee Tak[1] | Jin-Min Jung[1,2] | Jong Lyul Lee[1,2] | Chan Wook Kim[1,2] | Young-Chang Ah[3] | Seok-Soon Kim[3] | Ik Jun Moon[4] | Yong Sik Yoon[1,2]

[1]Asan Institute for Life Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea | [2]Division of Colon and Rectal Surgery, Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea | [3]BRPHARM Co., Ltd, Seoul, South Korea | [4]Department of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea

Correspondence: Yong Sik Yoon (yoonys@amc.seoul.kr)

Received: 17 June 2024 | **Revised:** 6 August 2024 | **Accepted:** 5 September 2024

Funding: This study was supported by grants (2020IF0009 and 2024IP0034) from the Asan Institute for Life Sciences, Asan Medical Center, Seoul, South Korea.

Keywords: dermal | epidermal | hyaluronic acid | polynucleotide | trans-epidermal water loss

ABSTRACT

Background: The epidermal barrier acts as a defense against external agents as well as helps to maintain body homeostasis. Polynucleotides (PN), exogenous DNA fragments, promote wound repair through their stimulatory and anti-inflammatory effects. Recent findings indicate a synergistic effect of PN and hyaluronic acid (HA) combinations in regulating inflammation and promoting cell proliferation. This study aims to elucidate the effects of PN and HA on repairing the epidermal barrier following its disruption by tape stripping (TS) in a mouse model.

Materials and Methods: After disrupting the epidermal barrier using TS, a formulation containing PN (14 mg/mL) and HA (6 mg/mL) was applied. Trans-epidermal water loss (TEWL) was measured at 0, 3, 6, 24, 48, and 72 h. Mice were euthanized after the final application at 72 h, and tissue samples were analyzed for epidermal/dermal thickness, neutrophil infiltration, and filaggrin expression.

Results: We observed a significant reduction in TEWL in the PN+HA group compared to that in the control group (20.8 ± 0.5 vs. 43.7 ± 0.5 g/m^{2}h at 72 h, $p < 0.05$), indicating an improvement in barrier function. Histological evaluation showed decreased epidermal and dermal thickening in the PN+HA group compared to that in the control group (epidermal: 29.4 ± 2.2 vs. 57.9 ± 3.5 μm; dermal: 464.8 ± 25.9 vs. 825.9 ± 44.8 μm, both $p < 0.05$). Additionally, neutrophil infiltration in the dermis was significantly reduced, and filaggrin protein levels were significantly higher in the PN+HA group compared to those in the control group (4.8 ± 0.4 vs. 21.1 ± 3.3 for neutrophils; 0.84 ± 0.04 vs. 0.42 ± 0.03 for filaggrin, both $p < 0.05$).

Conclusion: These results suggest that PN+HA may be an effective therapeutic strategy for repairing skin barrier damage.

1 | Introduction

The epidermal barrier function is critical for maintaining proper cutaneous hydration and protecting inner body homeostasis. When the epidermis is damaged, skin dehydration occurs, compromising its ability to regenerate. Thus, providing an adequately moist environment is considered the basis of modern wound care. An appropriate level of hydration not only accelerates wound healing but also brings about excellent cosmetic outcomes. Therefore, in a state where the wound is subjected to dehydration, a dressing agent that effectively supplies moisture is utilized.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Author(s). *Skin Research and Technology* published by John Wiley & Sons Ltd.

Hyaluronic acid (HA) is an anionic, non-sulfated glycosaminoglycan that is abundant in the extracellular matrix (ECM) [1], and is known to affect cutaneous wound healing by regulating inflammation and tissue remodeling [2]. HA is synthesized by fibroblasts, keratinocytes, periodontal ligament cells, cementoblasts, and osteoblasts. As a complex polysaccharide composed of amino acids and uronic acids, HA is a large molecule with a molecular weight ranging from 5000 to 20 000 000, formed by alternating chains of N-acetylglucosamine and glucuronic acid. Moreover, HA and its derivatives are known to promote cutaneous regeneration and are widely used as raw materials in wound dressings that create a moist environment [3].

Polynucleotide (PN) is a DNA biopolymer primarily extracted from the gonads of salmonid fish [4], but it can also be extracted from plants and yeast [5, 6]. The biopolymer binds large amounts of water to form a gel that provides continuous hydration and replenishment of viscosity. As a raw material for medical devices, PN is used in cosmetic fillers or joint cavity injections. Previous studies have demonstrated that PN modulates inflammation and improves wound healing in oral mucositis. Moreover, PN has regenerative and stimulatory properties, which are crucial in the management of oral mucositis in a clinical setting [7]. Additionally, PN plays an important role in the pathogenesis of ischemic stroke by suppressing neuroinflammation caused by ischemia/reperfusion injury [8].

Clinical trials in patients with knee osteoarthritis have demonstrated that PN+HA injections are well-tolerated and effective in reducing joint pain. Combining these injections is more effective in alleviating pain and improving wound contraction [9]. Furthermore, PN+HA has demonstrated greater efficacy than HA alone in promoting human fibroblasts' proliferation and increasing ECM production [9–11]. PN+HA exhibits excellent biocompatibility and induces tissue regeneration. The combination is more effective than PN alone in stimulating the growth of primary gingival fibroblasts and promoting wound healing, making it a promising candidate for supportive therapy to promote soft tissue healing in the oral cavity [12].

To our knowledge, no reports have documented the effects of a gel formulation containing PN and HA on cutaneous regeneration or skin barrier damage recovery. In this study, we evaluated the effect of PN+HA on wound healing and attested to a synergistic effect of PN and HA.

2 | Materials and Methods

2.1 | Animals and Reagents

This study was reviewed and approved by the Institutional Animal Care and Use Committee (No. 2022-02-327) of Asan Institute for Life Sciences, Asan Medical Center. The committee abides by the Institute of Laboratory Animal Resource guide. Five-week-old male BALB/c-nu mice were purchased from Orient Bio (Orient Bio Inc, Seongnam, South Korea). The maintenance of the animals was conducted on a 12 h light/dark cycle along with standard conditions of temperature and humidity of 22°C ± 1°C and 50% ± 10%, respectively. Acclimatization was performed for at least a week before studies, with nutrition based on a sterilized pellet-based diet. PN+HA (PN 14 mg and HA 6 mg per 1 mL saline) was supplied by BRPHARM (Wonju, South Korea). NDA PLUS was purchased from B&S Meditech (Seoul, South Korea) and used as a positive control.

2.2 | Induction of Mechanical Injury and Topical Application

Tape stripping (TS) is employed in numerous studies related to skin to examine the pathophysiology of various dermatologic conditions, including inflammatory and neoplastic diseases [13–15]. In addition, the TS technique is widely employed to evaluate the penetration of topically applied drugs.

After 1 week of acclimatization, the mice were divided into four groups of six mice each: normal (non-TS group), control (TS stimulated and saline-treated group), PN+HA (TS stimulated and PN+HA treated group), and NDA PLUS (TS stimulated and NDA PLUS treated group). Excluding the normal group, the mice were anesthetized with 1.4% isoflurane and 100% oxygen, the mechanical injury was induced by TS, using 50 strokes of transparent tape (Scotch, 3 M, Saint Paul, MN, USA) across their backs. For each stripping, a fresh piece of tape was lightly pressed onto the back and pulled off. The trans-epidermal water loss (TEWL) values were determined before TS, right after TS (0 h), and at 3, 6, 24, 48, and 72 h. Baseline TEWL was approximately 7 g/m^{2}h. After TS, visible wounds were noted and TEWL values ranged from 90 to 100 g/m^{2}h. The successfully modeled 18 mice were randomly divided into a normal saline-applied control group, PN+HA group, and NDA PLUS group. One hour after modeling, the first treatment was administered to mice using sterile skimmed cotton balls to apply saline, PN+HA, and NDA PLUS. The mice were treated every 24 h after the initial treatment for 3 days. In the normal group, mice were observed until the end of the experiment without receiving any treatment. Two hours after the final treatment, all mice were euthanized, and tissue samples were collected for histological analysis. Primarily, comparisons were made with the normal group and the TS-induced control group to confirm that the skin barrier damage model was well-induced. Subsequent comparisons were then made between the other groups and the control group.

2.3 | Evaluation of the Macroscopic Skin Lesion Score

On day 3, mice were sacrificed, and the skin lesion score was evaluated based on the following four symptoms: erythema, hemorrhage, oozing, and crust. Each symptom was graded as 0–3 (none, 0; mild, 1; moderate, 2; and severe, 3) and the final score was calculated by summing the individual grades. The total score ranged from 0 to 12.

2.4 | Measurement of TEWL

The barrier function of the skin was evaluated using the TEWL assay with GPSkin (GPOWER, Seoul, South Korea) on the back

16000846, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/srt.70068 by Yong Sik Yoon - University Of Ulsan , Wiley Online Library on [20/09/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

skin of each mouse. Water loss was recorded in g/m^{2}h. TEWL measurements were collected in triplicate, and the average values were used for graphs. The rate of barrier recovery was calculated using the following formula: [1 − (TEWL at indicated time − average TEWL of the normal group at the indicated time)/(TEWL immediately after TS − average TEWL of the normal group at the indicated time)] × 100.

2.5 | Histopathological Examination

The dorsal skin tissues from the mice were collected and fixed in 10% neutral buffered formalin at room temperature for 24 h before being embedded in paraffin. Slides of each section were stained with hematoxylin and eosin (H&E) to visualize epidermal and dermal thickness and to count the number of neutrophils per high-power field (×400). Immunohistochemical staining for filaggrin was performed to evaluate the effects on skin barrier function-related proteins in the epidermis. Skin sections were stained with filaggrin antibody (1: 500, orb10662, biobyt, Durham, NC, USA) in accordance with the manufacturer's instructions. Slides were scanned using a VS200 scanner (Olympus, Hamburg, Germany) and analyzed by Olyvia 3.3 software (Olympus). All results were quantified as the average of five randomly selected fields per section.

2.6 | Statistical Analysis

Results were analyzed using SPSS21 software. All experiments were conducted independently in triplicate. Results were presented as mean ± standard deviation (SD). Analysis of variance was performed to analyze the statistically significant differences between the groups. A p-value of <0.05 was considered statistically significant.

3 | Results

3.1 | Effect of PN+HA on Body Weight Change and Skin Lesion Score

After inducing superficial wounds using TS, PN+HA was administered topically on the dorsal skin. Body weight changes in the mice of each group over 3 days are displayed in Figure 1A. The body weight of each group increased steadily over time, with no significant difference between the groups. Photographs were taken every 24 h following the disruption. As illustrated in Figure 1B, symptoms such as TS-induced erythema, hemorrhage, oozing, and crust were observed on the dorsal skin of mice. The skin lesion score increased to 7.4 ± 0.5 in the control group and was significantly lower in the PN+HA group compared to that in the NDA PLUS group (control vs. PN+HA: 3.4 ± 0.5, $p < 0.05$; control vs. NDA PLUS: 5.1 ± 0.5, $p < 0.05$; PN+HA vs. NDA PLUS, $p < 0.05$; Figure 1C).

3.2 | Effect of PN+HA on TS-Induced TEWL

Variations in skin barrier function were evaluated by measuring TEWL in the TS-induced mouse model. Immediately after damage, TEWL was significantly increased in the TS-induced group compared to the normal group (control: 99.4 ± 1.1 g/m^{2}h vs. the normal group: 7.6 ± 0.5 g/m^{2}h, $p < 0.05$; Figure 2A). TEWL decreased in the PN+HA and NDA PLUS groups compared to its value in the control group at 3, 6, 24, 48, and 72 h. At 72 h, TEWL in the PN+HA group was significantly lower values than that in the control group (control: 43.7 ± 0.5 g/m^{2}h vs. PN+HA: 20.8 ± 0.5 g/m^{2}h, $p < 0.05$; control vs. NDA PLUS: 24.6 ± 1.3 g/m^{2}h, $p < 0.05$; PN+HA vs. NDA PLUS, $p < 0.05$). Moreover, the rate of barrier recovery increased in the PN+HA and NDA PLUS groups compared to the control group at 6, 24, 48, and 72 h (Figure 2B). At 24 h, the rate of barrier recovery of each group was compared to the control group, with the PN+HA group exhibiting significant recovery in skin barrier function than that observed in the NDA PLUS group (control: 22.8% ± 0.4% vs. PN+HA: 56.7% ± 1.2%, $p < 0.05$; control vs. NDA PLUS: 45.3% ± 0.8%, $p < 0.05$; PN+HA vs. NDA PLUS, $p < 0.05$).

3.3 | Effect of PN+HA on TS-Induced Epidermal and Dermal Thickness

To investigate the effects of PN+HA on the skin in the TS-induced mouse model, dermal and epidermal thicknesses were determined using H&E staining. In the control group, both the epidermis and dermis were abnormally thickened, and parakeratosis was prominently observed (Figure 3A). Despite a slightly thick, smooth epidermis, the overall structural integrity of the skin of the PN+HA group closely resembled that of normal skin upon gross microscopic examination. Conversely, the NDA PLUS group exhibited multiple hyperplastic dermal papillae and did not differ grossly from the control group. Overall, the PN+HA group maintained a better dermal structure on gross examination compared to that in the other groups. The epidermal thickness significantly increased in the control group due to TS (control: 57.9 ± 3.5 μm vs. the normal group: 17.6 ± 1.6 μm, $p < 0.05$; Figure 3B), but this increase was significantly reduced by the topical application of PN+HA and NDA PLUS (control vs. PN+HA: 29.4 ± 2.2 μm, $p < 0.05$; control vs. NDA PLUS: 36.5 ± 2.5 μm, $p < 0.05$; PN+HA vs. NDA PLUS, $p = 0.064$). Additionally, dermal thickness was significantly increased in the control group (control: 825.9 ± 44.8 μm vs. the normal group: 384.8 ± 33.0 μm, $p < 0.05$; Figure 3C), but this increase was significantly reduced by the topical application of PN+HA and NDA PLUS (control vs. PN+HA: 464.8 ± 25.9 μm, $p < 0.05$; control vs. NDA PLUS: 588.5 ± 23.9 μm, $p < 0.05$; PN+HA vs. NDA PLUS, $p < 0.05$).

3.4 | Effect of PN+HA on TS-Induced Neutrophil Infiltration

To investigate the anti-inflammatory effect of PN+HA, tissue sections were stained with H&E to examine neutrophil infiltration in skin wounds. The increase in inflammatory cells primarily consisted of neutrophils, with characteristic horseshoe-shaped or lobulated nuclei (Figure 4A, arrows). The number of neutrophils per high-power field in the dermis was significantly increased in the control group compared to the number in the normal group (control: 21.1 ± 3.3 vs. the normal group: 3.1 ± 0.6, $p < 0.05$;

16000846, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/srt.70068 by Yong Sik Yoon - University Of Ulsan , Wiley Online Library on [20/09/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

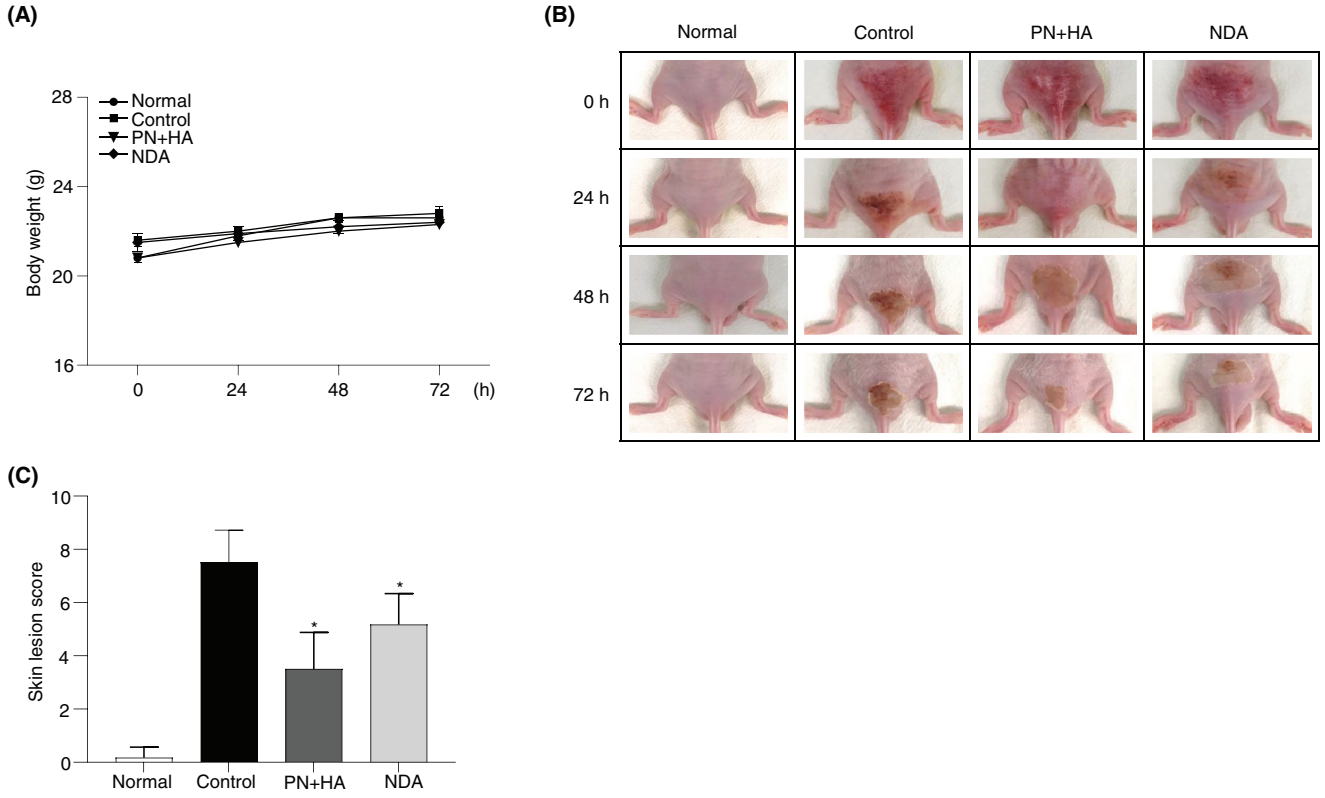


FIGURE 1 | Changes in body weight and skin wounds of mice. (A) Changes in body weight in each group during treatment. (B) Development of skin wounds in each group during treatment. Photos were obtained every 24 h after disruption. (C) Skin lesion score was quantified based on the erythema, hemorrhage, oozing, and crust. Data were presented as mean ± SD ($n = 6$). *$p < 0.05$ compared with the control group. Normal: non-TS group; Control: TS stimulated and saline-treated group; PN+HA: TS stimulated and PN+HA treated group; NDA: TS stimulated and NDA PLUS treated group. PN+HA, polynucleotide and hyaluronic acid; SD, standard deviation; TS, tape stripping.

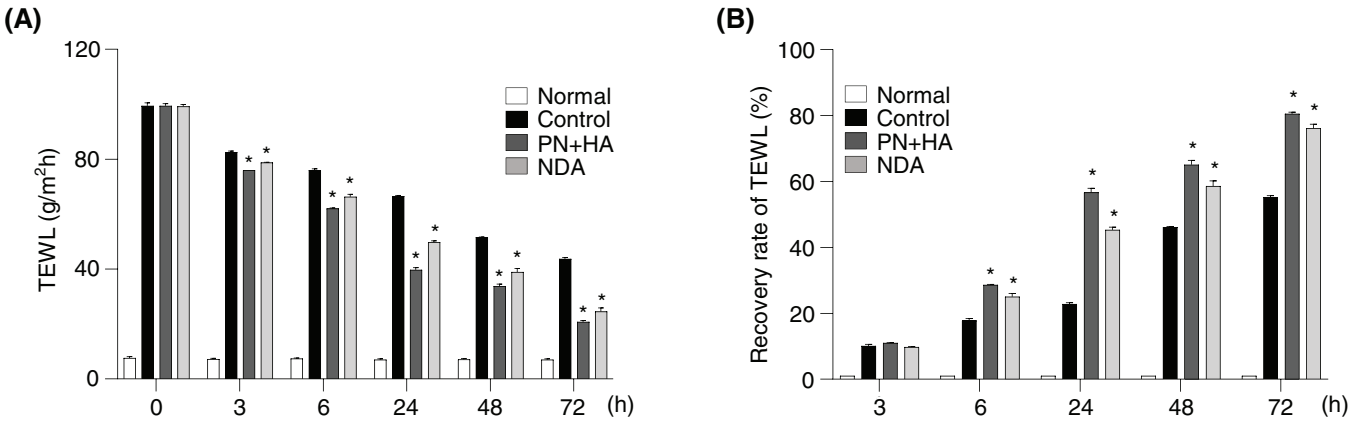


FIGURE 2 | Effect of PN+HA on TS-induced TEWL. TEWL was measured on the backs of the mice as described in the methods. TEWL values were measured at 0, 3, 6, 12, 24, 48, and 72 h. (A) TEWL. (B) The rate of barrier recovery. Data were presented as mean ± SD ($n = 6$). *$p < 0.05$ compared with the control group. Normal: non-TS group; Control: TS stimulated and saline-treated group; PN+HA: TS stimulated and PN+HA treated group; NDA: TS stimulated and NDA PLUS treated group. PN+HA, polynucleotide and hyaluronic acid; SD, Standard deviation; TEWL, trans-epidermal water loss; TS, tape stripping.

Figure 4B). Moreover, the number of neutrophils significantly decreased in the PN+HA and NDA PLUS groups compared to the number in the control group (control vs. PN+HA: 4.8 ± 0.4, $p < 0.05$; control vs. NDA PLUS: 6.2 ± 1.5, $p < 0.05$; PN+HA vs. NDA PLUS, $p = 0.614$).

3.5 | Effect of PN+HA on TS-Induced Filaggrin Expression

Immunohistochemistry staining for filaggrin was used to evaluate the effects of PN+HA on skin barrier function-related proteins

16000846, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/srt.70068 by Yong Sik Yoon - University Of Ulsan , Wiley Online Library on [20/09/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

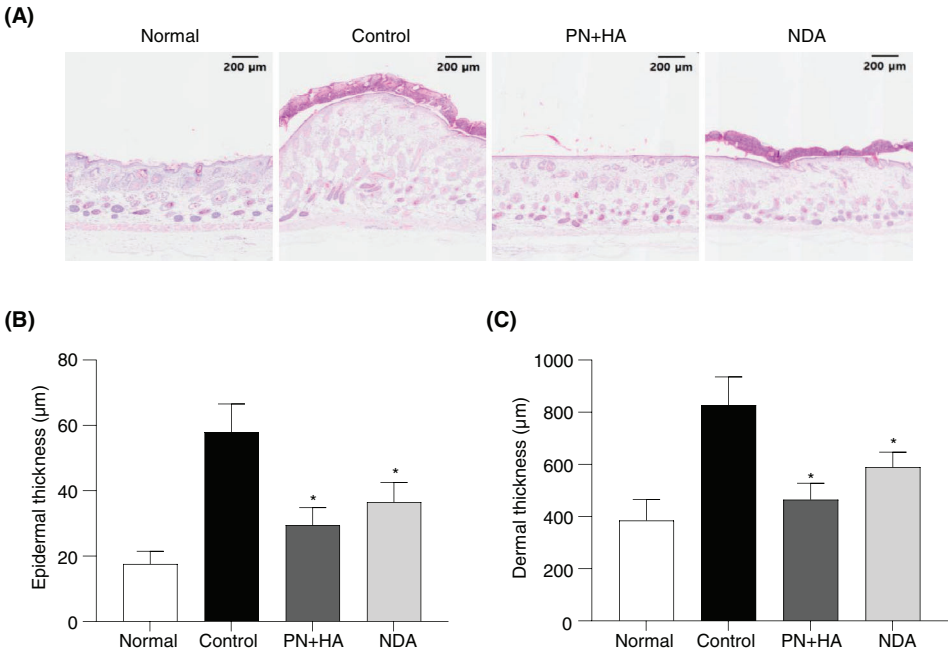


FIGURE 3 | Effect of PN+HA on TS-induced epidermal and dermal thicknesses. Dorsal skin was harvested at 72 h, and skin sections were stained with hematoxylin and eosin. (A) Pictures were acquired under a 40× objective. (B and C) The epidermal and dermal thickness of dorsal skin were quantified as means in randomly selected five fields per section. Data were presented as mean ± SD ($n = 6$). *$p < 0.05$ compared with the control group. Normal: non-TS group; Control: TS stimulated and saline-treated group; PN+HA: TS stimulated and PN+HA treated group; NDA: TS stimulated and NDA PLUS treated group. H&E, hematoxylin and eosin; PN+HA, polynucleotide and hyaluronic acid; SD, standard deviation; TS, tape stripping.

in the epidermis. In the normal group, the epidermal layer was intact, densely keratinized, and exhibited a dark brown stain (Figure 5A). The expression level of filaggrin was significantly decreased in the control group compared to the normal group (control: 0.42 ± 0.03 vs. the normal group: 1.00 ± 0.04, $p < 0.05$; Figure 5B). However, the filaggrin expression was significantly increased in the PN+HA group compared to the expression in the control group (control vs. PN+HA: 0.84 ± 0.04, $p < 0.05$; control vs. NDA PLUS: 0.66 ± 0.02, $p < 0.05$; PN+HA vs. NDA PLUS, $p < 0.05$).

4 | Discussion

In the present study, we observed significant improvements in the PN+HA treated group compared to the control group after TS in mice, including reduced TEWL, decreased epidermal and dermal thickening, reduced neutrophil infiltration, and higher filaggrin protein levels.

Perturbation of epidermal barrier function can be observed in various clinical conditions including repeated trauma (picking, rubbing, etc.) as well as acute and chronic dermatitis. Restoration of the epidermal barrier function is thus critical for the skin recovery. Prolonged impairment of the epidermal barrier function not only causes discomfort but may also increase the risk of both cutaneous infection and chemical irritation, which can interfere with the adequate healing process. Topical glucocorticoids and antihistamines are commonly used to treat skin wounds [16–18]. Although these treatments can alleviate epidermal barrier impairment by promoting wound healing and regeneration, they also have significant side effects. The skin condition can significantly deteriorate once the drug is discontinued, rendering it unsuitable for long-term use [19, 20]. Although maintaining a moist environment is a simple and effective strategy to promote the restoration of the epidermal barrier and wound healing, active research is underway to identify compounds that can further accelerate this process.

Furthermore, HA plays a pivotal role in maintaining the epidermal barrier function of the skin due to its unique ability to bind and retain water molecules, significantly contributing to skin hydration and turgidity. The current literature highlights the potential of HA in facilitating the repair processes necessary for skin barrier recovery [21, 22]. Additionally, a recent study by Park et al. demonstrated that HA can improve skin barrier function by upregulating tight junction-related proteins in keratinocytes [23]. HA has long been recognized as a scaffold due to its good tolerability and ease of use in different fields, including skin diseases [24, 25].

PN is emerging and is gaining recognition as a key agent in enhancing the epidermal barrier function of the skin. Acting as building blocks of DNA and RNA, PN is known to be an adenosine A2A receptor agonist [26]. PN can stimulate skin rejuvenation and repair by promoting cellular growth, differentiation, and migration. All of these processes are essential for maintaining the integrity of the skin barrier [27]. Furthermore, PN has also been demonstrated to boost the production of ECM components such as collagen and elastin, thereby improving skin elasticity, hydration, and overall texture [28].

By combining the skin hydration and excellent permeability of HA with the skin regeneration ability of PN, the PN-HA

16000846, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/srt.70068 by Yong Sik Yoon - University Of Ulsan , Wiley Online Library on [20/09/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

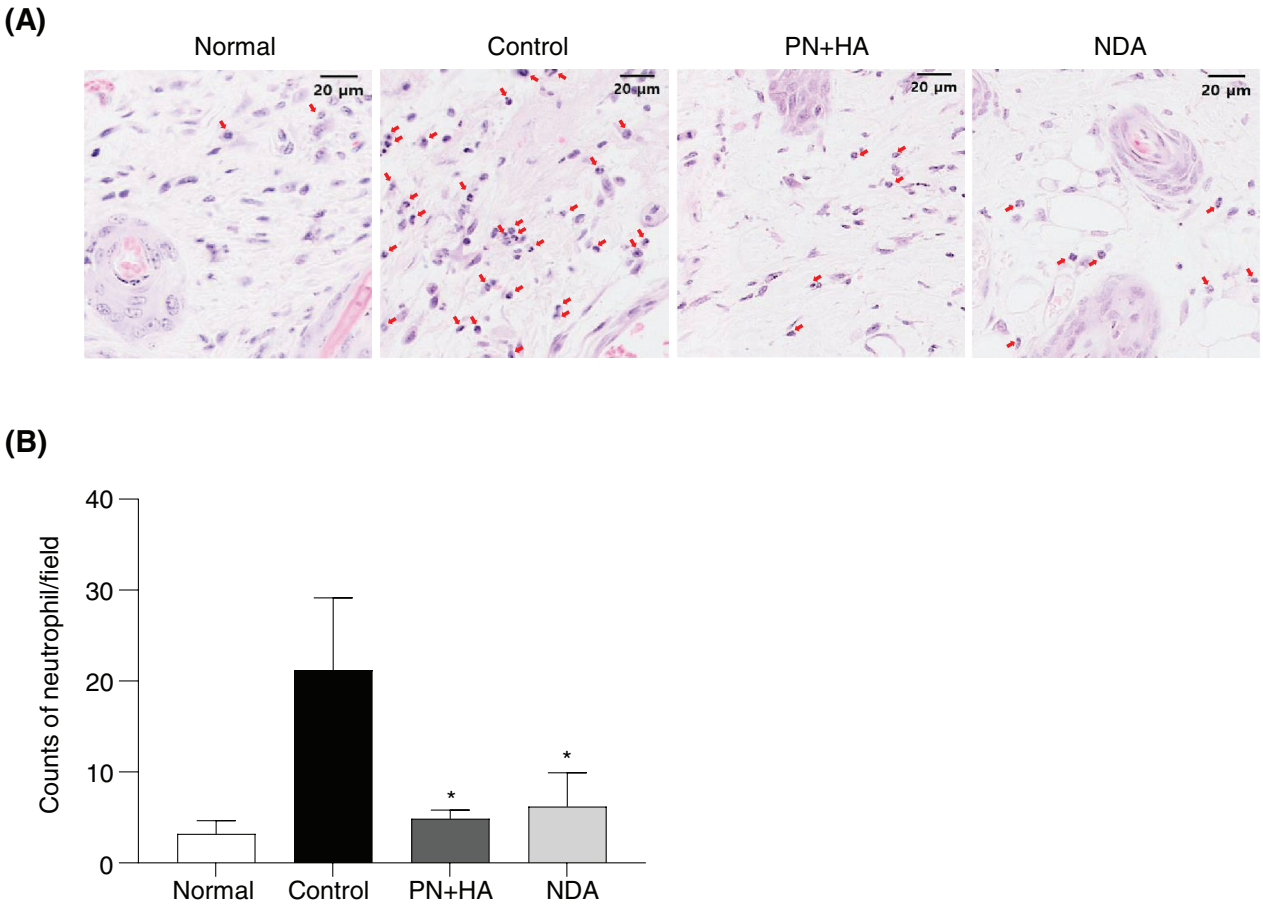


FIGURE 4 | Effect of PN+HA on TS-induced neutrophil infiltration. Neutrophil infiltration was confirmed via hematoxylin and eosin staining. (A) Pictures were obtained under a 400× objective. (B) Neutrophil infiltration was quantified as means in randomly selected five fields per section. Data were presented as mean ± SD ($n = 6$). *$p < 0.05$ compared with the control group. Normal: non-TS group; Control: TS stimulated and saline-treated group; PN+HA: TS stimulated and PN+HA treated group; NDA: TS stimulated and NDA PLUS treated group. PN+HA, polynucleotide and hyaluronic acid; SD, standard deviation; TS, tape stripping.

composite filler offers excellent biocompatibility. This filler induces tissue regeneration, fills intradermal spaces, provides hydration, viscoelasticity, and plumps up tissues [11, 29]. The combination of HA and PN could be a promising skin moisturizer, that not only promotes drug penetration but also increases its retention in the skin, playing a role in skin wound therapy and skin care. NDA PLUS, which includes *Alchemilla vulgaris* extract, glycerol, honey, and green tea extract, was selected as a positive control because it is one of the most commonly used commercial ointments for wound care.

The TS experiment is a model of mechanically induced acute irritant dermatitis and can disrupt the integrity of the epidermal barrier in animal models. Although TS was carried out consistently across all mice to induce uniform damage, slight differences were observed among the individual mice. To compensate for this difference, we randomly divided them into groups. Compared to the NDA PLUS group, mice treated with PN+HA demonstrated significantly reduced dermal thickness, with rapid recovery of the epidermis in terms of parakeratosis and hyperplasia.

Additionally, TS aggravates defects in skin barrier function and leads to the release of inflammatory cytokines that play major roles in driving the immune response to antigens [30]. Neutrophils are key players in immunity and influence inflammatory and immune reactions through the production of numerous cytokines and chemokines [31]. PN+HA reduced the expression of pro-inflammatory neutrophilic infiltration compared to that observed with NDA PLUS, but no significant difference between the PN+HA and the NDA PLUS groups was observed. Filaggrin is an essential structural protein for skin barrier function and is down-regulated under inflammatory conditions, leading to the disruption of the skin barrier [32, 33]. TS reduces the production of skin barrier-related proteins such as filaggrin in keratinocytes [15, 34]. In our immunohistochemical assay, filaggrin protein expression was significantly increased following topical application of PN+HA compared to NDA PLUS, demonstrating superior regeneration of the skin by PN+HA.

This study has two limitations. First, the anti-inflammatory and proliferation effects were evaluated through neutrophil infiltration, epidermal/dermal thickness, and filaggrin expression. However, these findings are limited by the use of surrogate endpoints, which can lack direct clinical relevance. Second, the observed effects of the combination of PN + HA cannot be determined is due to which of them because of the lack of PN and HA only groups.

16000846, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/srt.70068 by Yong Sik Yoon - University Of Ulsan , Wiley Online Library on [20/09/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

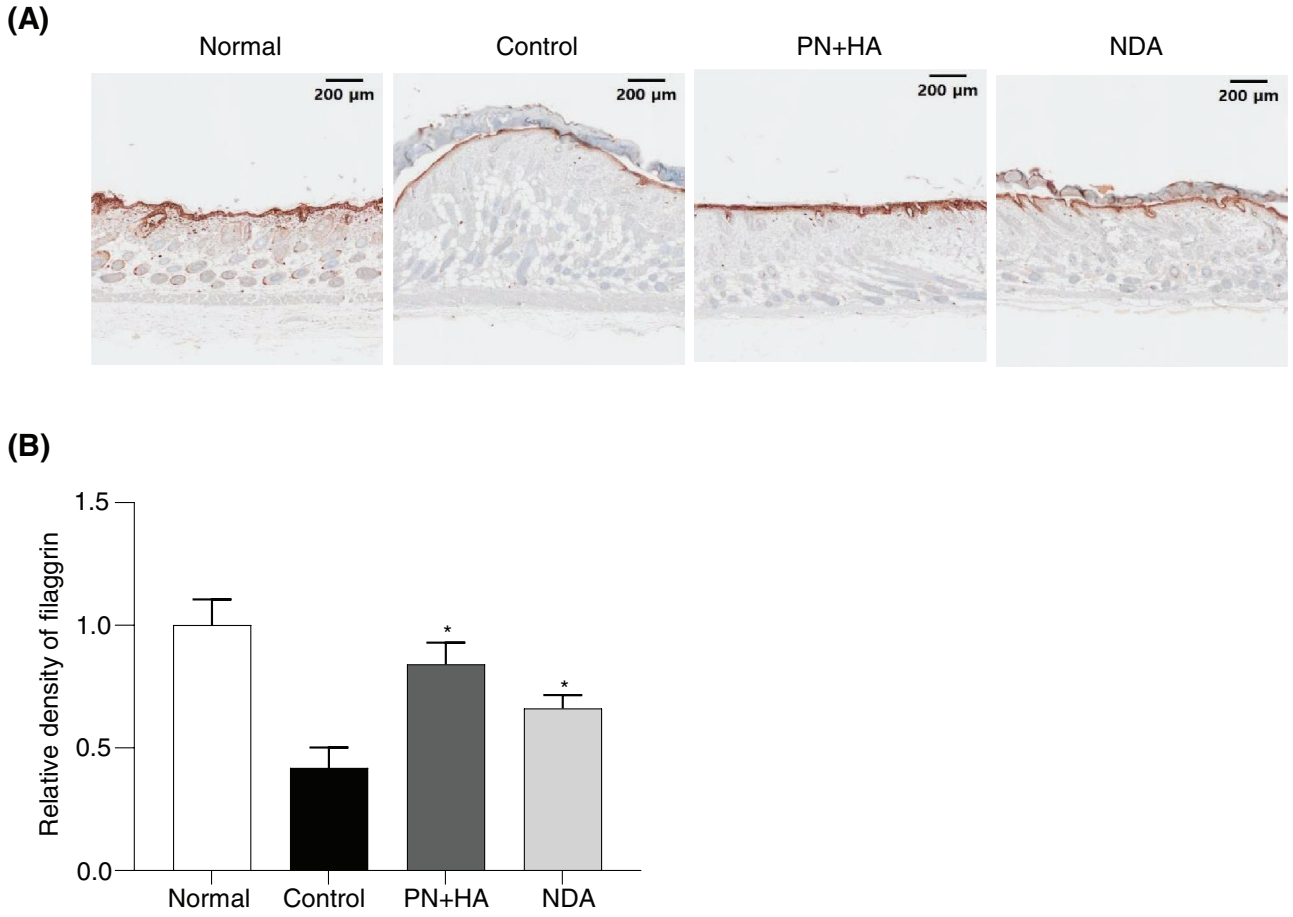


FIGURE 5 | Effect of PN+HA on TS-induced filaggrin expression. Dorsal skin was harvested at 72 h, and skin sections were stained for filaggrin using immunohistochemistry. (A) Pictures were obtained under a 40× objective. (B) The density of filaggrin of dorsal skin was qualified as means in randomly selected five fields per section using the ImageJ program. Data were presented as mean ± SD ($n = 6$). *$p < 0.05$ compared with the control group. Control, TS stimulated and saline-treated group; NDA, TS stimulated and NDA PLUS treated group; Normal, non-TS group; PN+HA, TS stimulated and PN+HA treated group. PN+HA, polynucleotide and hyaluronic acid; SD, standard deviation; TS, tape stripping.

In conclusion, our findings indicate that the combination of PN and HA attenuated epidermal hyperplasia, regulated inflammation, and facilitated the recovery of the epidermal barrier in a TS mouse model. Further research, along with intensive in vitro experimental and clinical studies are needed to explore the therapeutic potential of HA in combination with PN in the treatment of cutaneous wounds and dermatitis.

Consent

All authors have read and approved the manuscript.

Conflicts of Interest

Young-Chang Ah and Seok-Soon Kim are employed by BRPHARM Co., Ltd. The rest of the authors state no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

References

1. R. C. Gupta, R. Lall, A. Srivastava, and A. Sinha, "Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory," *Frontiers in Veterinary Science* 6 (2019): 192.

2. M. N. Leite and M. A. C. Frade, "Efficacy of 0.2% Hyaluronic Acid in the Healing of Skin Abrasions in Rats," *Heliyon* 7, no. 7 (2021): e07572.

3. A. Pilloni, M. A. Rojas, C. Trezza, et al., "Clinical Effects of the Adjunctive use of Polynucleotide and Hyaluronic Acid-Based Gel in the Subgingival Re-Instrumentation of Residual Periodontal Pockets: A Randomized, Split-Mouth Clinical Trial," *Journal of Periodontology* 94, no. 3 (2023): 354–363.

4. M. Cavallini, E. Bartoletti, L. Maioli, et al., "Consensus Report on the Use of PN-HPT (Polynucleotides Highly Purified Technology) in Aesthetic Medicine," *Journal of Cosmetic Dermatology* 20, no. 3 (2021): 922–928.

5. K. S. Lee, S. Lee, H. Wang, et al., "Analysis of Skin Regeneration and Barrier-Improvement Efficacy of Polydeoxyribonucleotide Isolated From Panax Ginseng (C.A. Mey.) Adventitious Root," *Molecules (Basel, Switzerland)* 28, no. 21 (2023): 7240.

6. S. Segarra, I. Bošnjak, I. Mioč, et al., "Yeast-Derived Nucleotides Enhance Fibroblast Migration and Proliferation and Provide Clinical Benefits in Atopic Dermatitis," *International Journal of Molecular Sciences* 25, no. 5 (2024): 2890.

16000846, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/srt.70068 by Yong Sik Yoon - University Of Ulsan , Wiley Online Library on [20/09/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

7. G. Picciolo, F. Mannino, N. Irrera, et al., "PDRN, a Natural Bioactive Compound, Blunts Inflammation and Positively Reprograms Healing Genes in an "In Vitro" Model of Oral Mucositis," *Biomedicine & Pharmacotherapy* 138 (2021): 111538.

8. S. Jo, A. Baek, Y. Cho, et al., "Therapeutic Effects of Polydeoxyribonucleotide in an In Vitro Neuronal Model of Ischemia/Reperfusion Injury," *Scientific Reports* 13, no. 1 (2023): 6004.

9. D. Dallari, G. Sabbioni, N. Del Piccolo, et al., "Efficacy of Intra-Articular Polynucleotides Associated With Hyaluronic Acid Versus Hyaluronic Acid Alone in the Treatment of Knee Osteoarthritis: A Randomized, Double-Blind, Controlled Clinical Trial," *Clinical Journal of Sport Medicine* 30, no. 1 (2020): 1–7.

10. G. De Caridi, M. Massara, I. Acri, et al., "Trophic Effects of Polynucleotides and Hyaluronic Acid in the Healing of Venous Ulcers of the Lower Limbs: A Clinical Study," *International Wound Journal* 13, no. 5 (2016): 754–758.

11. J. H. Kim, T. R. Kwon, S. E. Lee, et al., "Comparative Evaluation of the Effectiveness of Novel Hyaluronic Acid-Polynucleotide Complex Dermal Filler," *Scientific Reports* 10, no. 1 (2020): 5127.

12. M. T. Colangelo, S. Belletti, P. Govoni, S. Guizzardi, and C. Galli, "A Biomimetic Polynucleotides-Hyaluronic Acid Hydrogel Promotes Wound Healing in a Primary Gingival Fibroblast Model," *Applied Sciences (Basel)* 11, no. 10 (2021): 4405.

13. A. J. Hughes, S. S. Tawfik, K. P. Baruah, E. A. O'Toole, and R. F. L. O'Shaughnessy, "Tape Strips in Dermatology Research," *British Journal of Dermatology* 185, no. 1 (2021): 26–35.

14. M. Denda, L. C. Wood, S. Emami, et al., "The Epidermal Hyperplasia Associated With Repeated Barrier Disruption by Acetone Treatment or Tape Stripping Cannot be Attributed to Increased Water Loss," *Archives of Dermatological Research* 288, no. 5-6 (1996): 230–238.

15. M. Demerjian, M. Q. Man, E. H. Choi, et al., "Topical Treatment With Thiazolidinediones, Activators of Peroxisome Proliferator-Activated Receptor-Gamma, Normalizes Epidermal Homeostasis in a Murine Hyperproliferative Disease Model," *Experimental Dermatology* 15, no. 3 (2006): 154–160.

16. S. K. Stacey and M. McEleney, "Topical Corticosteroids: Choice and Application," *American Family Physician* 103, no. 6 (2021): 337–343.

17. D. E. Cohen and N. Heidary, "Treatment of Irritant and Allergic Contact Dermatitis," *Dermatologic Therapy* 17, no. 4 (2004): 334–340.

18. J. I. Silverberg, A. Armstrong, A. Blauvelt, and K. Reich, "Assessment of Efficacy and Safety Outcomes Beyond Week 16 in Clinical Trials of Systemic Agents Used for the Treatment of Moderate to Severe Atopic Dermatitis in Combination With Topical Corticosteroids," *American Journal of Clinical Dermatology* 24, no. 6 (2023): 913–925.

19. A. B. Mehta, N. J. Nadkarni, S. P. Patil, K. V. Godse, M. Gautam, and S. Agarwal, "Topical Corticosteroids in Dermatology," *Indian Journal of Dermatology, Venereology and Leprology* 82, no. 4 (2016): 371–378.

20. U. Darsow, A. Wollenberg, D. Simon, et al., "ETFAD/EADV Eczema Task Force 2009 Position Paper on Diagnosis and Treatment of Atopic Dermatitis," *Journal of the European Academy of Dermatology and Venereology* 24, no. 3 (2010): 317–328.

21. V. Voinchet, P. Vasseur, and J. Kern, "Efficacy and Safety of Hyaluronic Acid in the Management of Acute Wounds," *American Journal of Clinical Dermatology* 7, no. 6 (2006): 353–357.

22. H. Roehrs, J. G. Stocco, F. Pott, G. Blanc, M. J. Meier, and F. A. Dias, "Dressings and Topical Agents Containing Hyaluronic Acid for Chronic Wound Healing," *Cochrane Database of Systematic Reviews (Online)* 7, no. 7 (2023): Cd012215.

23. H.-Y. Park, D.-K. Kweon, and J.-K. Kim, "Upregulation of Tight Junction-Related Proteins by Hyaluronic Acid in human HaCaT Keratinocytes," *Bioactive Carbohydrates and Dietary Fibre* 30 (2023): 100374.

24. F. Della Sala, A. Fabozzi, M. di Gennaro, et al., "Advances in Hyaluronic-Acid-Based (Nano) Devices for Cancer Therapy," *Macromolecular Bioscience* 22, no. 1 (2022): e2100304.

25. S. Vasvani, P. Kulkarni, and D. Rawtani, "Hyaluronic Acid: A Review on its Biology, Aspects of Drug Delivery, Route of Administrations and a Special Emphasis on its Approved Marketed Products and Recent Clinical Studies," *International Journal of Biological Macromolecules* 151 (2020): 1012–1029.

26. M. Galeano, G. Pallio, N. Irrera, et al., "Polydeoxyribonucleotide: A Promising Biological Platform to Accelerate Impaired Skin Wound Healing," *Pharmaceuticals (Basel)* 14, no. 11 (2021): 1103.

27. M. Yu and J. Y. Lee, "Polydeoxyribonucleotide Improves Wound Healing of Fractional Laser Resurfacing in Rat Model," *Journal of Cosmetic and Laser Therapy* 19, no. 1 (2017): 43–48.

28. A. M. O. Pratama and A. R. Muharram, "Salmon DNA Combination for Hypertrohic Scar Treatment With Diabetes Mellitus Type 2: A Case Report," *International Journal of Medical Science and Clinical Research Studies* 2, no. 8 (2022): 846–849.

29. A. Araco, F. Araco, and M. Raichi, "Clinical Efficacy and Safety of Polynucleotides Highly Purified Technology (PN-HPT®) and Cross-Linked Hyaluronic Acid for Moderate to Severe Nasolabial Folds: A Prospective, Randomized, Exploratory Study," *Journal of Cosmetic Dermatology* 22, no. 1 (2023): 146–155.

30. S. Jang, J. Ohn, J. W. Kim, et al., "Caffeoyl-Pro-His Amide Relieve DNCB-Induced Atopic Dermatitis-Like Phenotypes in BALB/c Mice," *Scientific Reports* 10, no. 1 (2020): 8417.

31. T. Ear and P. P. McDonald, "Cytokine Generation, Promoter Activation, and Oxidant-Independent NF-kappaB Activation in a Transfectable Human Neutrophilic Cellular Model," *BMC Immunology [Electronic Resource]* 9 (2008): 14.

32. A. D. Irvine, W. H. McLean, and D. Y. Leung, "Filaggrin Mutations Associated With Skin and Allergic Diseases," *New England Journal of Medicine* 365, no. 14 (2011): 1315–1327.

33. W. Peng and N. Novak, "Pathogenesis of Atopic Dermatitis," *Clinical and Experimental Allergy* 45, no. 3 (2015): 566–574.

34. Y. Chen, X. Li, X. Gan, et al., "Fucoidan From Undaria Pinnatifida Ameliorates Epidermal Barrier Disruption via Keratinocyte Differentiation and CaSR Level Regulation," *Marine Drugs* 17, no. 12 (2019): 660.

16000846, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/srt.70068 by Yong Sik Yoon - University Of Ulsan , Wiley Online Library on [20/09/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License