의학박사 학위논문

폴리뉴클레오타이드-히알루론산 하이드로겔의 창상 회복 효과

Enhanced Wound Healing Induced by Polynucleotide-Hyaluronic Acid Hydrogel in a Mouse Model of Mechanical Injury

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의 학 과

정 진 민

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이 논문을 의학박사 학위 논문으로 제출함

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Abstract

Background

Wound healing involves a complex interplay of cell growth, migration, and matrix remodeling. 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.

Purpose

The aim of this study is to evaluate the effect of PN+HA to modulate inflammatory responses and enhance cellular proliferation during the wound healing process.

Methods

Twenty-four mice were acclimatized for one week before experimentation. Six mice served as the normal group, while the remaining 18 were subjected to tape stripping (TS) to induce mechanical epidermal injury. These injured mice were randomly divided into three groups: one treated with normal saline, another with PN+HA hydrogel, and the third with NDA. Topical applications were performed at 1, 24, 48, and 72 hours after TS. Trans-epidermal water loss (TEWL) was measured at 0, 3, 6, 24, 48, and 72 hours. Mice were euthanized 2 hours after the final application at 72 hours, and tissue samples were analyzed for neutrophil count, epidermal/dermal thickness, and filaggrin density.

Results

TS effectively induced a mechanical injury model, with a significant TEWL increase in all experimental groups. The PN+HA group exhibited the fastest TEWL recovery, higher than the control group by day 3 (20.8 ± 0.5 g/m² h versus 43.7 ± 0.5 g/m² h, $p < 0.05$). Histological analysis revealed that the PN+HA group had lower neutrophil counts (4.8 ± 0.4 versus 21.1 ± 3.3, $p < 0.05$) and reduced epidermal/dermal thickness (epidermal: 29.4 ± 2.2 μm versus 57.9 ± 3.5 μm; dermal: 464.8 ± 25.9 μm versus 825.9 ± 44.8 μm, both $p < 0.05$) compared to the control group, indicating an anti-inflammatory effect. Furthermore, the PN+HA group exhibited significantly higher filaggrin density compared to the control group (84.1 ± 3.5 versus 41.6 ± 2.7, $p < 0.05$), indicating superior epidermal differentiation.

Conclusion

PN+HA reduced inflammation and promoted epidermal differentiation, thereby accelerating wound healing in a model of mechanical epidermal injury.

Keywords: polynucleotide, hyaluronic acid, wound healing, proliferation, anti-inflammatory effect

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1. Introduction

Wound healing progresses through a series of processes involving the activation of various cells. This process traditionally occurs in three stages: inflammation, new tissue formation, and remodeling. Inflammation begins immediately after tissue damage, with a platelet plug forming to achieve hemostasis and a fibrin matrix providing a scaffold for cell infiltration. Neutrophils and macrophages then migrate to the wound. In the new tissue formation stage, cell proliferation occurs, and angiogenesis is stimulated by vascular endothelial growth factor and fibroblast growth factor, replacing the fibrin matrix with granulation tissue and providing a foundation for keratinocyte movement. Re-epithelialization is facilitated by growth factors like epidermal growth factor and fibroblast growth factor, promoting keratinocyte proliferation and migration. During remodeling, most cells undergo apoptosis, leaving a collagen-rich matrix that is actively restructured. Wound healing is an orchestrated integration of biological and molecular events involving cell growth, migration, and matrix deposition. Various agents have been studied to enhance wound healing by influencing these processes.

Along with the enhanced scientific interest in the roles of deoxyribonucleic acid (DNA) extracts in cell proliferation, the properties of extracellular nucleotides and nucleosides as regenerative agents in wound healing have been revisited.[1,2] Extracellular nucleotides have been shown to activate purinergic receptors, specifically P_2 receptors, which are expressed on various cell types involved in wound healing, including keratinocytes, fibroblasts, and immune cells.[3-5] The activation of P_2 receptors leads to a cascade of cellular responses that promote tissue regeneration and repair. Extracellular nucleotides also promote angiogenesis via P_2Y receptors and VEGF upregulation, modulate inflammation through P_2X-induced pro-inflammatory cytokine release and P_2Y-mediated anti-inflammatory effects, thus balancing the wound environment for optimal healing.[5-7]

Polynucleotides (PNs), a mixture of deoxyribonucleotides, were first isolated from human placenta in the 1990s using an innovative extraction technique. This technique facilitated the isolation of a final compound from human placenta that exhibited specific therapeutic activity. Subsequently, a series of experimental studies were published, demonstrating the cell proliferation effects of these exogenous nucleotides.[8,9] These findings highlighted the potential of PNs in various biomedical applications, particularly in areas requiring enhanced cell proliferation and tissue regeneration. In the early 2000s, the successful extraction of PNs from salmon sperm significantly increased their availability. This advancement facilitated numerous studies elucidating the biological mechanisms and therapeutic potential of these bioactive compounds.[10] PNs were extracted and purified from the sperm of Salmon trout or Chum Salmon at high temperature through a process. The extraction process ensures a high

degree of purity (>95%) and eliminates pharmacologically active proteins and peptides, reducing the risk of immunological side effects. It comprises a mixture of deoxyribonucleotide polymers with chain lengths between 50 and 2000 base pairs, and molecular weights ranging from 50 to 1500 kDa.[11] PNs degradation yields active nucleotides (purines and pyrimidines) that exert biological effects via two mechanisms: adenosine (A_2) receptor signaling and the DNA salvage pathway. A_2 receptors modulate inflammation, angiogenesis, and cell growth, with PNs acting as pro-drugs interacting with A_2 receptors.[12,13] In damaged tissues, where de novo DNA synthesis is impaired, PNs provide nucleotide precursors for the salvage pathway, facilitating DNA synthesis and cell proliferation.[14]

PNs exhibit diverse pharmacological properties that contribute to their stimulatory effect on cell growth, particularly in the realms of wound healing, angiogenesis, and anti-inflammatory activity. PNs enhance wound healing by promoting fibroblast proliferation and migration through A_2A receptor signaling, facilitating granulation tissue formation and re-epithelialization.[15,16] They also foster angiogenesis by upregulating VEGF expression and stimulating endothelial cell proliferation, thereby promoting neovascularization.[17,18] Furthermore, PNs exert anti-inflammatory effects by interacting with A_2A receptors, decreasing levels of pro-inflammatory cytokines like TNF-α and IL-6, while elevating anti-inflammatory mediators like IL-10. This immune modulation prevents excessive inflammation, a potential hindrance to healing, and fosters a regenerative microenvironment conducive to tissue repair.[13]

Hyaluronic acid (HA) is an anionic, non-sulfated glycosaminoglycan and is known for its remarkable ability to retain moisture and promote wound healing.[19] It plays a crucial role in wound healing by facilitating cell migration, proliferation, and tissue regeneration.[20] Due to these properties, HA is widely used in various medical and cosmetic applications, including dermal fillers and wound dressings. Recently, there has been growing interest in the study of PN+HA combinations, as these mixtures have shown potential to enhance the wound healing process by synergistically regulating inflammation and promoting cellular proliferation. PN+HA has demonstrated greater efficacy than HA alone in promoting human fibroblasts proliferation and increasing extracellular matrix production.[21-23] Clinical trials in patients affected by 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.[21]

This study will explore the potential of PN+HA combinations to promote wound healing. Through a mouse model of mechanical epidermal injury induced by tape stripping, we aim to evaluate the effect of PN+HA to modulate inflammatory responses and enhance cellular proliferation during the wound healing process.

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, 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 ± 1 ℃ and 50 ± 10%, respectively. Acclimatization was performed at least a week before studies while the nutrition was based on a sterilized pellet-based diet. PN+HA (HA 6 mg and PN 14 mg per 1 mL saline) was supplied by BRPHARM (Wonju-si, Gangwon-do, Korea). NDA PLUS was purchased from B&S Meditech (Seoul, 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 several dermatologic conditions, including epidermal injury, inflammatory, and neoplastic diseases.[24-26] In addition, the TS technique has been widely used to evaluate the penetration of topically applied drugs.

After 1 week of acclimatization, 6 mice were assigned to the normal group, while the remaining 18 mice were subjected to tape stripping and then randomly divided into 3 groups of 6 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, after 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, 3M, Saint Paul, MN, USA) across their back. For each stripping, a fresh piece of tape was lightly pressed onto the back and pulled off. The transepidermal 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 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 last treatment, the mice in all groups 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.

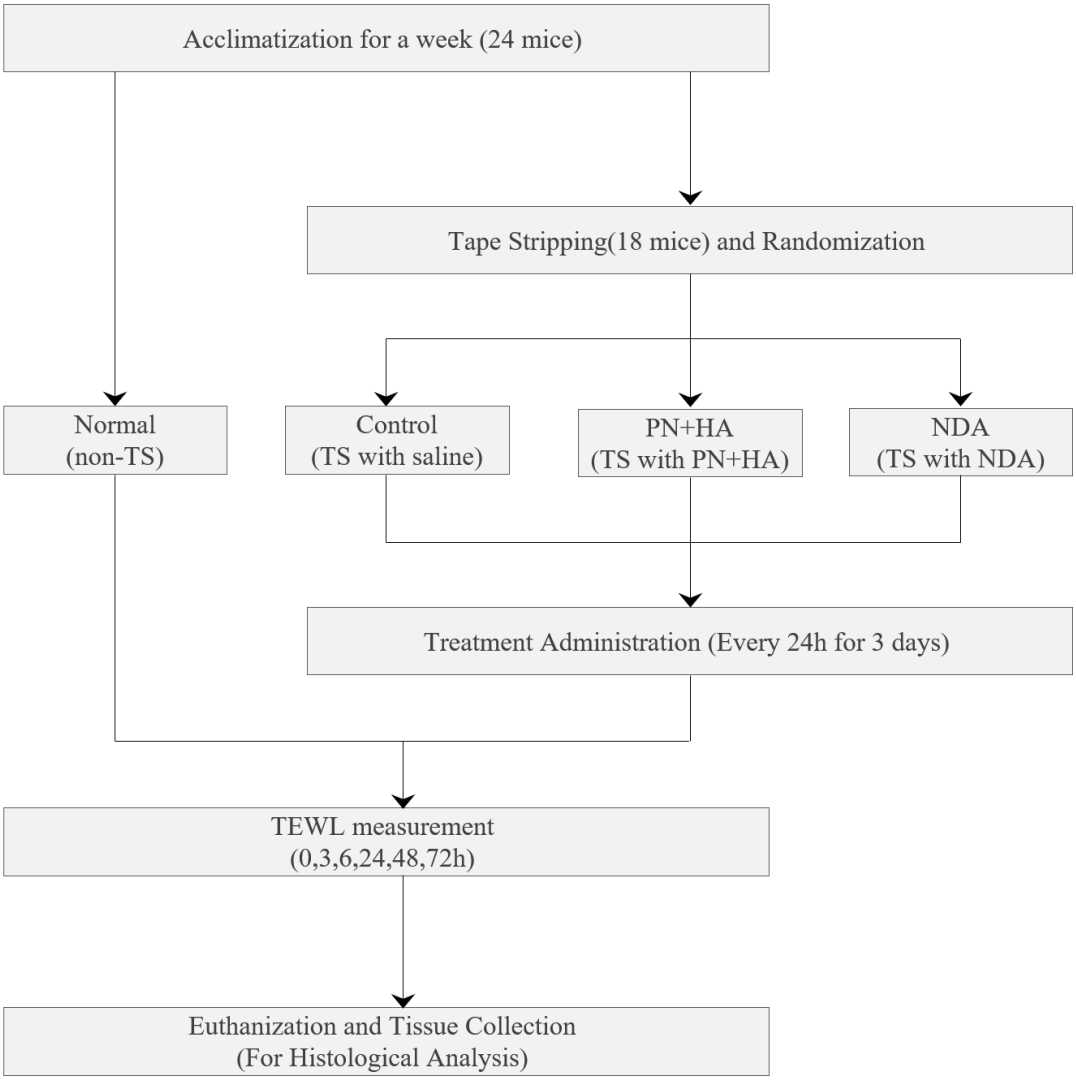

Figure 1. Flow diagram, A detailed illustration of the study design, including the randomization process, intervention groups, and outcome assessments

2.3 Measurement of TEWL

The barrier function of the skin was evaluated using the TEWL assay with GPSkin (GPOWER, Seoul, Korea) on the back skin of each mouse. Water loss was recorded in g/m^2h. TEWL measurements were collected in triplicate, and the average values were used for graphs. The percentage 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.

2.4 Evaluation of the severity of dermatitis

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

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 (x400). Immunohistochemical staining for filaggrin was performed to evaluate the effects on epidermal differentiation. 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 SPSS 21 software. All experiments were conducted independently in triplicates. Results are 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 Effects of PN+HA on body weight change and dermatitis 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 2A. The body weight of each group increased steadily over time, with no significant difference between the groups. Photographs were obtained every 24 h after disruption. As illustrated in Figure 2B, symptoms such as

TS-induced erythema, hemorrhage, crust, and dryness were observed on the dorsal skin of mice. The dermatitis score increased to 7.4 ± 0.5 in the control group and significantly decreased 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 2C).

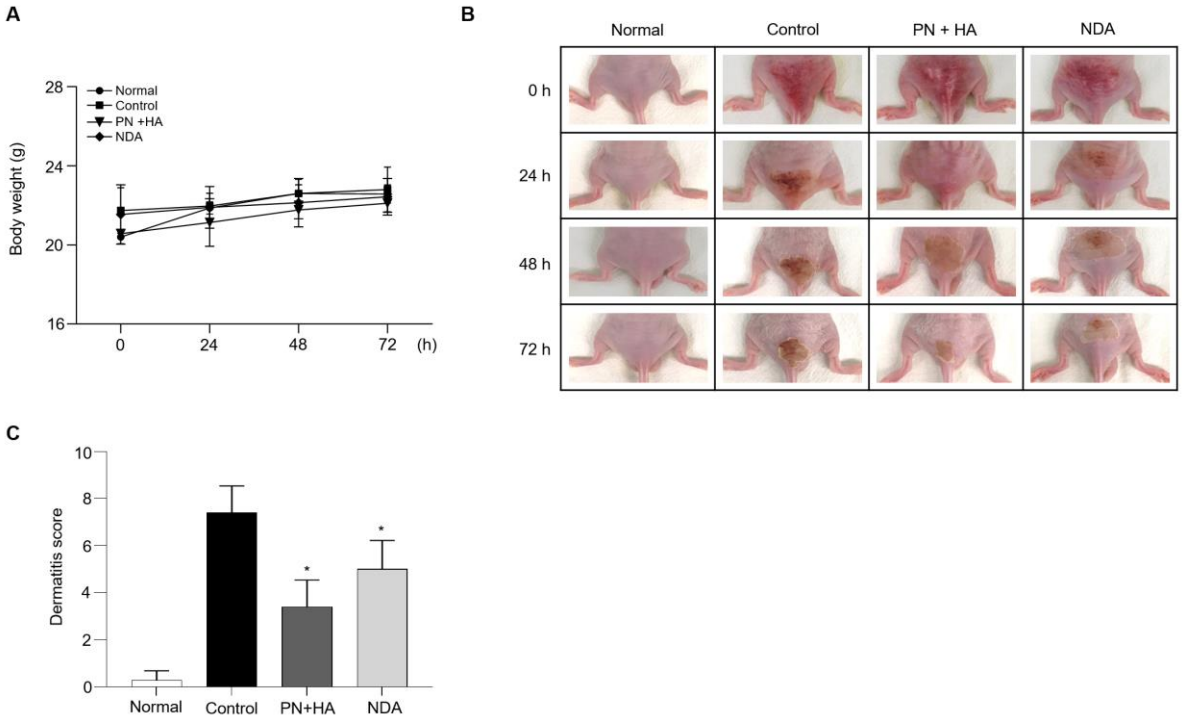

Figure 2. Changes in body weight and skin wounds of mice

(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) Dermatitis score.

Data are presented as mean ± SD (n = 6). *$p < 0.05$ versus the control group.

3.2 Effects 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 that in the normal group (control: 99.4 ± 1.1 g/m² h vs. the normal group: 7.6 ± 0.5 g/m² h, $p < 0.05$; Figure 3A). 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 demonstrated significantly lower values than those of the control group (control: 43.7 ± 0.5 g/m² h vs. PN+HA: 20.8 ± 0.5 g/m² h, $p < 0.05$; control vs. NDA PLUS: 24.6 ± 1.3 g/m² h, $p < 0.05$; PN+HA vs. NDA PLUS, $p < 0.05$). Moreover, the recovery rate of TEWL increased in the PN+HA and NDA PLUS groups

compared to that in the control group at 6, 24, 48, and 72 h (Figure 3B). At 24 h, the recovery rate of each group was compared to the control group, with the PN+HA group exhibiting significant better 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, $p < 0.05$).

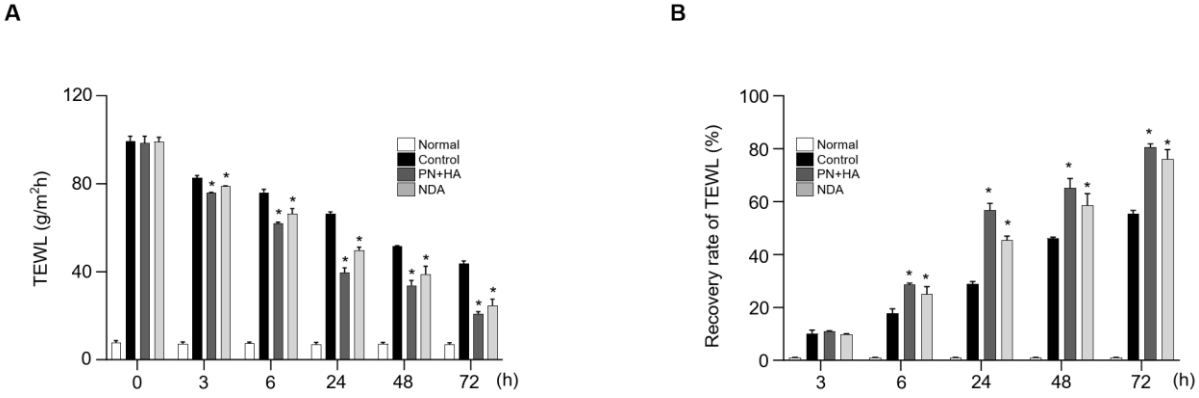

Figure 3. Effects of PN+HA on TEWL

(A) TEWL. (B) Recovery of TEWL. Data are presented as mean ± SD (n = 6). *$p < 0.05$ versus the control group.

3.3 Effects of PN+HA on TS-induced epidermal and dermal thickness

To investigate the effects of PN+HA on the skin of the TS-induced mouse model, the dermal and epidermal thicknesses were determined using H&E staining. The epidermis and dermis were abnormally thickened in the control group, and parakeratosis was prominently observed (Figure 4A). The overall structural integrity of the skin of the PN+HA group closely resembled that of normal skin upon gross microscopic examination, despite a slightly thick, smooth epidermis. Conversely, the NDA PLUS group demonstrated multiple hyperplastic dermal papillae and was not grossly different 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 4B), 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 4C), which 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 ± 2.5 μm, $p < 0.05$; PN+HA vs. NDA PLUS, $p < 0.05$).

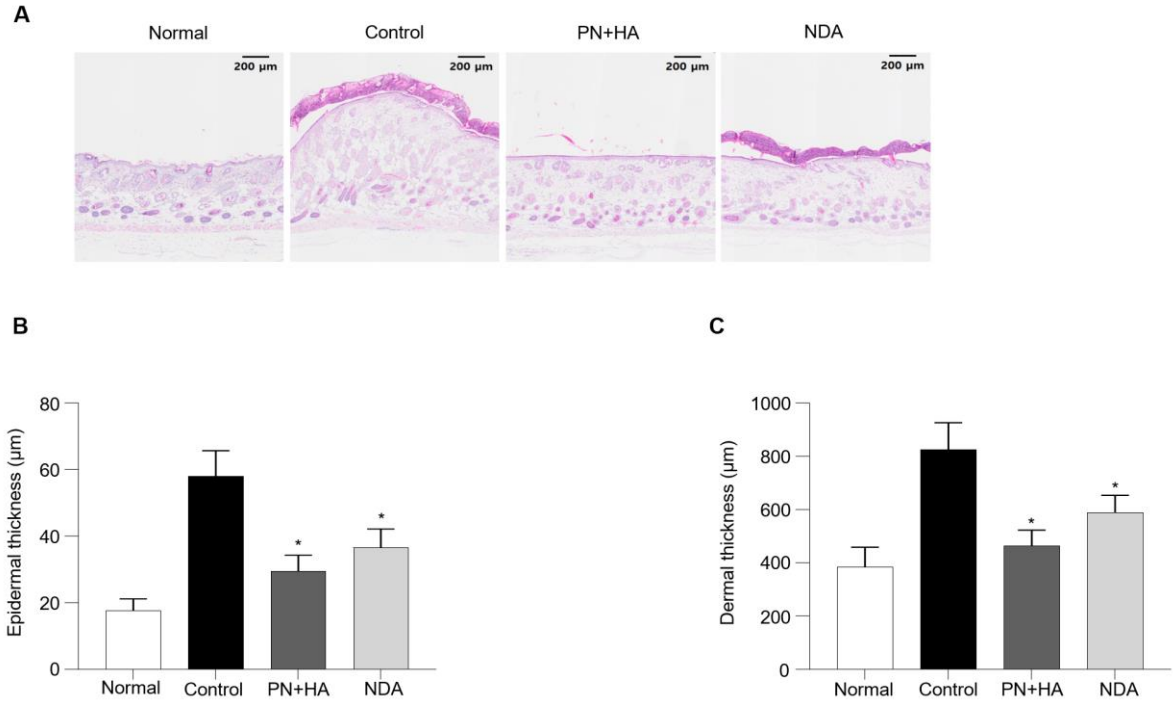

Figure 4. Effects of PN+HA on epidermal and dermal thicknesses

Dorsal skin is harvested at 72 h, and skin sections are prepared and stained with hematoxylin and eosin. (A) Pictures are acquired under a 40× objective. (B, C) The epidermal and dermal thickness of dorsal skin is quantified as means in randomly selected five fields per section. Data are presented as mean ± SD (n = 6). *$p < 0.05$ versus the control group.

3.4 Effects 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 consisted primarily of neutrophils, with characteristic horseshoe-shaped or lobulated nuclei (Figure 5A, 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, $p < 0.05$; Figure 5B). 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, $p = 0.604$).

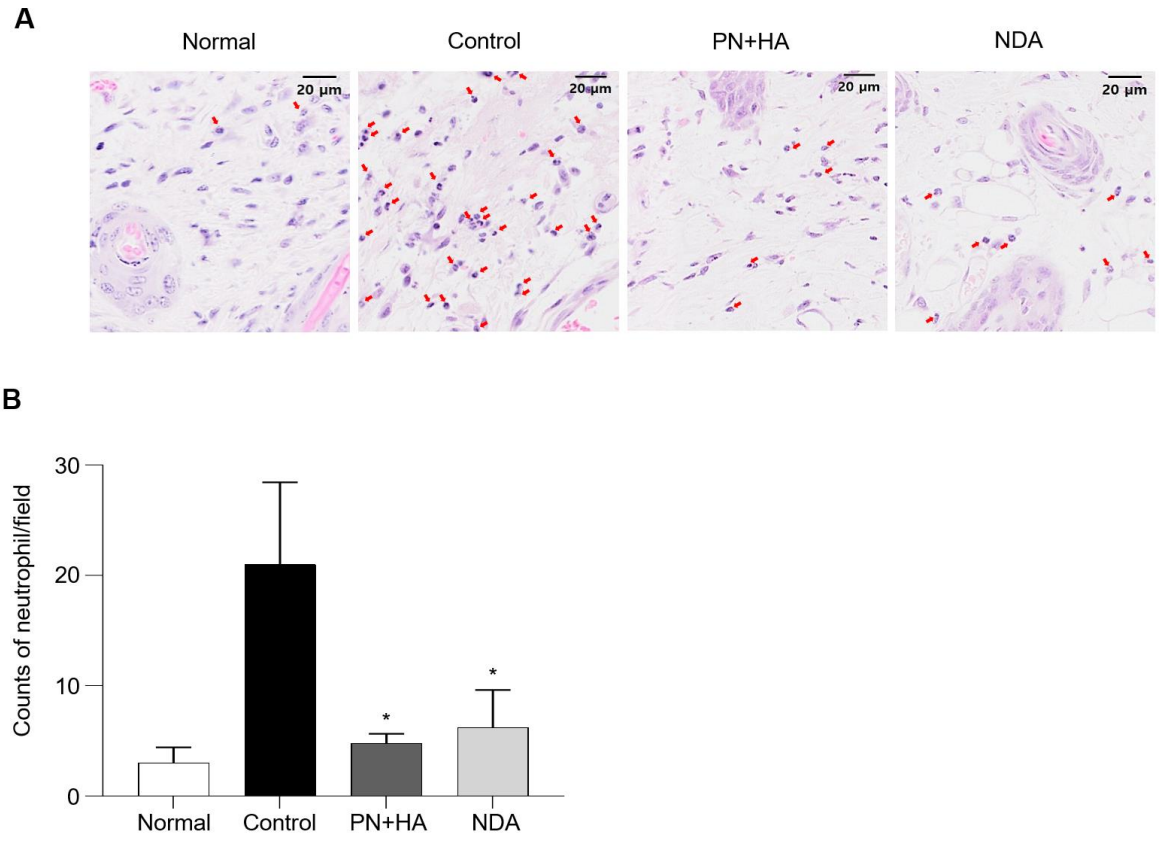

Figure 5. Effects of PN+HA on neutrophil infiltration

Neutrophil infiltration was confirmed via hematoxylin and eosin staining. (A) Pictures are obtained under a 400× objective. (B) Neutrophil infiltration is quantified as means in randomly selected five fields per section.

3.5 Effects of PN+HA on TS-induced filaggrin expression

Immunohistochemistry staining for filaggrin was used to evaluate the effects of PN+HA on epidermal differentiation. In the normal group, the epidermal layer was intact, densely keratinized, and exhibited a dark brown stain (Figure 6A). The expression level of filaggrin was significantly decreased in the control group compared to that in the normal group (control: 41.6 ± 2.7 vs. the normal group: 100.1 ± 4.7, $p < 0.05$; Figure 6B). However, the expression was significantly increased in the PN+HA group compared to the expression in the control group (control vs. PN+HA: 84.1 ± 3.5, $p < 0.05$; control vs. NDA PLUS: 65.9 ± 2.7, $p < 0.05$; PN+HA vs. NDA PLUS, $p < 0.05$).

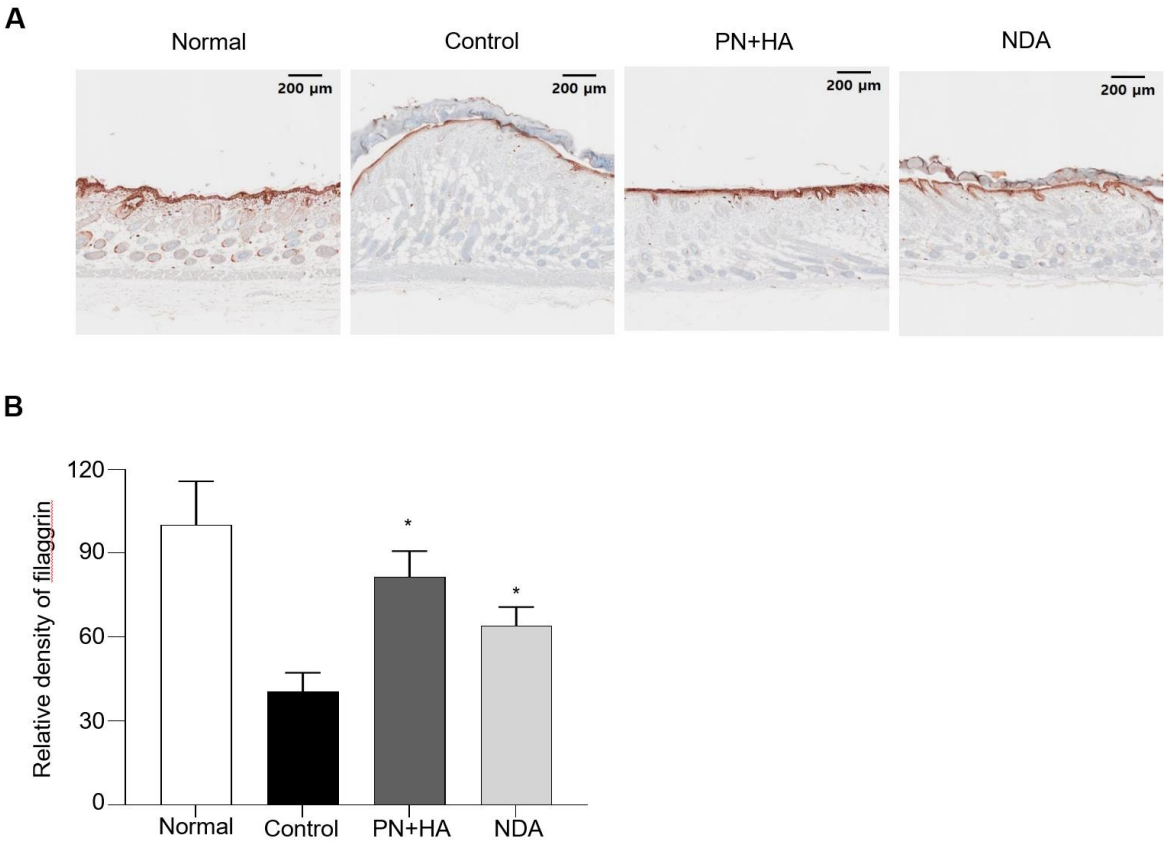

Figure 6. Effects of PN+HA on TS-induced filaggrin expression

Dorsal skin is harvested at 72 h, and skin sections are prepared and stained for filaggrin using immunohistochemistry. (A) Pictures are obtained under a 40× objective. (B) Filaggrin density of dorsal skin is quantified as the means in randomly selected five fields per section and analyzed using the ImageJ program. Data are presented as mean ± SD (n = 6). *$p < 0.05$ versus the control group.

	Normal	**Control**	**PN+HA**	**NDA**
Epidermal thickness (μm)	17.6	57.9	29.4	36.5
Dermal Thickness (μm)	384.8	825.9	464.8	588.5
Neutrophil infiltration (counts/HPF)	3.1	21.1	4.8	6.2
Filaggrin density	100.1	41.6	84.1	65.9

Table 1. Histopathological evaluation compared between each treatment modality

4. Discussion

Mechanical injury triggers the wound healing process, which begins with an initial inflammatory response. It is followed by neovascularization, leading to the formation of granulation tissue and a new epithelial layer. Eventually, scar formation occurs. The scar then contracts and undergoes remodeling.[27] However, disruption of the skin barrier due to injury can result in infection and delayed wound healing. Various local treatments have been developed to enhance skin barrier recovery. Among these treatments, extracellular nucleotides have been shown to stimulate cell proliferation and activity.[28,29] Notably, PNs, extracted from salmon sperm, have demonstrated promising results in enhancing wound healing by promoting fibroblast proliferation, VEGF expression, and angiogenesis[10]. Additionally, PNs have exhibited effectiveness in promoting cell proliferation and activity in various inflammatory conditions, such as osteoarthritis, tendinitis, and dental.[30-32] The present study aimed to evaluate the efficacy of a mixture of PNs and HA, which serves as a scaffold, in promoting wound healing.

Firstly, our study demonstrated that PN+HA effectively reduces water loss across the stratum corneum. TEWL measures the amount of water that escapes through the stratum corneum to the outside of the skin.[33] An impaired skin barrier function results in increased water loss. TEWL quantifies the water loss, and higher TEWL values indicate poorer skin barrier function. TEWL is widely utilized as an indicator of skin barrier function in both in vivo and in vitro studies.[34] In our study, TEWL was highest immediately after TS and continuously decreased over the 3-day study period. The PN+HA group showed the most significant decrease, reaching the lowest levels by day 3. These results indicate that PN+HA is effective in promoting the recovery of skin barrier function.

In our study, the neutrophil count was the lowest in the group treated with PN+HA, suggesting that PN+HA effectively reduces excessive neutrophil infiltration and activation, thereby minimizing chronic inflammation and promoting more efficient wound healing. The key to effective healing and tissue normalization post-injury is the successful transition from the inflammatory phase to the proliferative phase.[35] During the early stages of the wound healing process, circulating neutrophils are recruited and massively accumulated, leading the initial defense against infection or tissue damage.[36] Subsequently, these neutrophils undergo apoptosis and are cleared by macrophages, which also play active roles in regulating cell proliferation and angiogenesis.[37] However, excessive neutrophil infiltration and activation can release cytokines or proteases and activate lymphocytes, leading to tissue damage, chronic inflammation, and hindering wound recovery.[38]

In the group treated with PN+HA, the filaggrin density was measured to be higher than in the control group. Filaggrin is known to increase in re-epithelialized epithelium.[39] As keratinocytes migrate from

the granular layer to the corneal layer, profilaggrin is expressed and subsequently degraded to form filaggrin.[40] This filaggrin then aggregates and binds to keratin filaments, transforming the cytoplasm into the main components of corneocytes. Thus, filaggrin serves as a marker of epidermal differentiation.[41,42] Moreover, the degradation of filaggrin produces compounds such as amino acids that help maintain skin moisture.[43] The higher levels of filaggrin observed in the PN+HA group suggest that PN+HA promotes epidermal differentiation and increases filaggrin production. The increased production of filaggrin enhances re-epithelialization and, through its degradation products, helps maintain skin moisture, thereby contributing to wound healing.

HA has long been recognized as a scaffold due to its good tolerability and ease of use in different fields, including skin diseases.[44,45] By combining the skin hydration and excellent permeability of HA with the regeneration ability of PN, the PN+HA composite filler offers excellent biocompatibility. The filler also induces tissue regeneration, fills intradermal spaces, provides hydration, viscoelasticity, and plumps up tissues.[23,46] The combination of PN and HA 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.

The components of NDA PLUS are Alchemilla vulgaris extract, glycerol, honey, and green tea extract. Additionally, NDA PLUS was selected as a positive control because it is one of the most commonly used commercial ointments for wound care. A previous study demonstrated that the herbal mixture containing A. vulgaris has been shown to shorten the healing period of full-thickness wounds more effectively than fusidic acid. Additionally, it has demonstrated the ability to promote migration and cell growth of keratinocytes, fibroblast, and endothelial cells, and to close the wound surface diameter faster than fusidic acid.[47] A study has reported increased cell proliferation of epithelial cells and myofibroblasts, and this mixture has been shown to quickly reduce the diameter of excisional skin lesions.[48] Clinically, it has shown a high rate of complete healing and relieved discomfort in cases of aphthous ulcers.[49]

In our study, we employed a mechanical epidermal injury model using TS. The TS method involves the repeated application and removal of adhesive tape to the skin, resulting in the removal of the stratum corneum and creating a superficial epidermal injury. A study reported that 15 tape stripping removed 25% of the epidermis, while 35 tape stripping removed 33% of the epidermis[50]. In our study, we performed 50 tape stripping, which likely resulted in an epidermal injury of at least 33%. Although TS was carried out consistently across all mice to induce uniform damage, slight differences were observed among the individual mice. This model is useful for studying the early phases of wound healing and the

epidermal barrier function. In contrast, the incisional wound model involves creating a surgical incision through the full thickness of the skin, which better simulates a surgical wound and allows for the study of deeper tissue repair processes, including dermal and subdermal healing. While the incisional wound model provides a more comprehensive understanding of the wound healing process, particularly in a clinical context, the tape stripping method is less invasive and simpler to perform.

While the TS model primarily induces superficial epidermal injuries, it provides valuable insights into the initial wound healing processes that can be relevant to surgical wounds. Surgical wounds, being full-thickness incisional injuries, involve more complex healing mechanisms that extend beyond the epidermis. Therefore, while our findings on the efficacy of PN+HA in promoting epidermal repair are promising, further research is needed to explore their effects on deeper tissue healing. However, there are several surgical areas where these findings could be applied. Several studies have demonstrated that harnessing the proliferative and angiogenic properties of stem cells can effectively promote the closure of anal fistulas[51]. Since PN+HA have also been found to promote proliferation and have anti-inflammatory effects, further research in this area would be beneficial. Additionally, it would be worthwhile to investigate the effects of PN+HA on superficial burns.

This study has several 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 synergistic effect of PN+HA was evaluated, but it was not compared to PN or HA alone, making it difficult to directly compare with existing treatments. Third, the differing viscosities of the administered treatments may have influenced the results.

5. Conclusion

Our findings indicate that the combination of PN and HA reduced inflammation and promoted epidermal differentiation, thereby accelerating wound healing in a model of mechanical epidermal injury. 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 surgical wound.

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국문요약

배경: 창상 치유는 세포 성장, 이동, 기질 재형성의 복잡한 상호작용을 포함한다. 폴리뉴클레오티드(PN)는 자극 및 항염증 효과를 통해 창상 치유를 촉진한다. 최근 연구에 따르면 PN과 히알루론산(HA)의 조합이 염증 조절과 세포 증식 촉진에 시너지 효과가 있는 것으로 나타났다. 본 연구의 목적은 창상 치유 과정에서 PN+HA가 염증 반응을 조절하고 세포 증식을 촉진하는 효과를 평가하는 것이다.

방법: 24마리의 쥐를 실험 전 1주일 동안 환경에 적응하는 기간을 가졌다. 6마리는 정상군으로 사용하고, 나머지 18마리는 기계적 표피 손상을 유도하기 위해 테이프 스트리핑(TS)을 실시했다. TS를 실시한 쥐들은 무작위로 세 그룹으로 나누어 각각 식염수, PN+HA, NDA로 처리하였다. 국소 도포는 TS 후 1, 24, 48, 72시간에 실시했다. 경표피 수분 손실(TEWL)은 0, 3, 6, 24, 48, 72시간에 측정했다. 72시간째 최종 도포 2시간 후 쥐들을 안락사 하였고, 조직 샘플을 채취해 호중구 수, 표피/진피 두께, 필라그린 밀도를 분석했다.

결과: TS는 효과적으로 기계적 손상 모델을 유도했으며, 모든 실험군에서 TEWL이 유의하게 증가했다. PN+HA군은 가장 빠른 TEWL 회복을 보였고, 3일째에는 대조군보다 높은 회복을 보였다 (20.8 ± 0.5 g/m² h 대 43.7 ± 0.5 g/m² h, $p < 0.05$). 조직학적 분석 결과, PN+HA군은 대조군에 비해 호중구 수(4.8 ± 0.4 대 21.1 ± 3.3, $p < 0.05$)와 표피/진피 두께(표피: 29.4 ± 2.2 μm 대 57.9 ± 3.5 μm; 진피: 464.8 ± 25.9 μm 대 825.9 ± 44.8 μm, 모두 $p < 0.05$)가 감소했으며, 이는 항염증 효과를 나타낸다. 또한, PN+HA군은 대조군에 비해 필라그린 밀도가 유의하게 높았고(84.1 ± 3.5 대 41.6 ± 2.7, $p < 0.05$), 이는 증가된 표피 분화를 나타낸다.

결론: PN+HA는 기계적 표피 손상 모델에서 염증을 억제하고 표피 분화를 촉진하여 창상 치유를 향상시켰다.