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Original Article

Comparative evaluation of hyaluronic acid-based dressing versus hydrocolloid dressing in rat dermal wound healing

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Background: Wound healing is a complex process influenced by a variety of environmental factors. Dressing materials play a critical role in creating barriers against contaminants, maintaining optimal moisture levels, and absorbing wound exudate. Therefore, selecting materials tailored to wound characteristics is crucial for enhancing outcomes. Hyaluronic acid (HA) is a natural biocompatible polymer that supports healing by regulating inflammation and promoting tissue repair. This study compared HA- and hydrocolloid-based hydrogels in a rat model to optimize wound care strategies.

Methods: Full-thickness dermal wounds (diameter, 8 mm) were created on the dorsal skin of 12 Sprague-Dawley rats under sevoflurane anesthesia. The wounds were treated with HA/silver sulfadiazine gel (group A), hydrocolloid gel (group B), or left untreated (control), all covered with a transparent dressing. Biopsy specimens on days 3, 7, and 21 were used to assess histological parameters: inflammatory cell infiltration, fibroblast infiltration, collagen deposition, neovascularization, and epithelial thickness, using a semi-quantitative scoring system. Histological analyses were conducted blindly, and statistical analyses were performed using the Kruskal-Wallis test ($p<0.05$).

Results: On day 3, group A showed significantly higher inflammatory cell infiltration and collagen deposition than other groups, indicating extracellular matrix formation. By day 7, angiogenesis was highest in group A, followed by group B and controls. By day 21, all wounds had completely healed. Epithelial layer thickness, reflecting inflammation and fibroblast maturity, was significantly higher in group A.

Conclusion: This study compared HA-based hydrogel and hydrocolloid-based dressings through histological analyses to elucidate wound healing mechanics. HA-based hydrogel dressings significantly enhanced wound recovery. However, generalizing these outcomes requires future studies to expand the range of effective wound treatment materials. These findings underscore the potential of HA-based dressings to enhance clinical outcomes in wound management, suggesting avenues for improving therapeutic strategies.

Abbreviations: ECM, extracellular matrix; HA, hyaluronic acid; H&E, hematoxylin and eosin

Keywords: Hyaluronic acid / Hydrocolloid dressing / Wound healing

INTRODUCTION

Wound healing is a complex and intricate process, yielding different outcomes based on even subtle environmental variations. Therefore, providing an ideal environment is crucial for healing.

Dressing materials, which act as barriers against external contaminants, effectively shielding the wound from microbial invasion and reducing the risk of secondary infections, play a key role in wound care [1]. The ideal material can create an optimal

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moisture balance around the wound site, preventing excessive dryness or moisture, both of which can impede healing. Additionally, dressings facilitate the absorption of wound exudate, the fluid that oozes from the wound, which helps maintain a clean environment conducive to healing.

By carefully selecting dressing materials that align with the specific needs of a given wound, such as size, depth, location, or type of injury, healing outcomes can be improved [1,2]. Ideally, these materials consist of biocompatible substances that prevent inflammation and rejection reactions in the wound tissue. Natural high-molecular-weight polymers, such as hyaluronic acid (HA), are preferred because of their ability to activate healing signals while maintaining a moist wound environment. HA is a glycosaminoglycan that plays a crucial role in wound healing by regulating inflammation and promoting tissue repair. Its use in dressings underscores its therapeutic benefits for optimizing the wound-healing process [3].

The present study aimed to investigate the effects of an HA-based dressing by creating full-thickness dermal wounds on the dorsal skin of rats, which is histologically similar to that of humans [4]. We compared the healing effects of HA-based dressings with those from hydrocolloid-based dressings as well as those from controls, without dressings.

METHODS

Preparation

This experimental study involved 12 Sprague-Dawley rats, approximately 8 weeks old; they were maintained under appropriate temperature and humidity conditions with food and water provided throughout a 7-day acclimatization period before the start of the experiment. The rats were housed individually in cages and randomly divided into three groups of four each. Prior to creating the dermal wound, each rat underwent induction of anesthesia via inhaled sevoflurane. The selected area was shaved using an electric shaver and the skin was disinfected using 10% povidone-iodine and 70% alcohol. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Wonkwang University Hospital University.

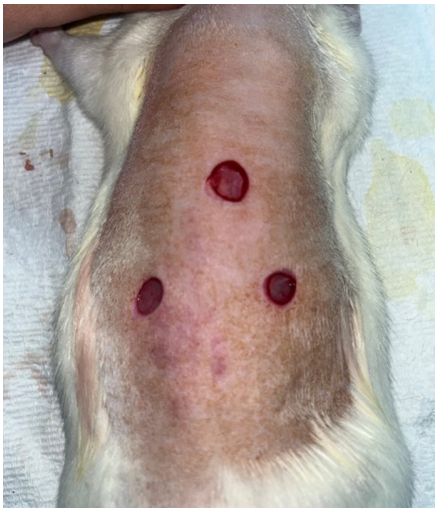

Fig. 1. Wound generation on back of rat with 8-mm-diameter biopsy punch.

Wound generation and care

Using an 8-mm diameter biopsy punch (Kai Medical), circular, full-thickness dermal wounds were created on the dorsal skin of the rats (Fig. 1). Adequate spacing was maintained between the wounds to avoid interference with the healing process of each wound, and minor bleeding and debris were washed off using a normal saline solution. Three different materials were prepared for application to the wounds: HA/silver sulfadiazine gel (Connettivina gel; Fidia Farmaceutici S.p.A.), hydrocolloid gel (Duoderm hydroactive gel; ConvaTec Inc.), and a transparent dressing (Tegaderm; 3M). Group A received an application of Connettivina gel sufficient to cover the wound, whereas group B received the same amount of Duoderm hydroactive gel. The control group did not receive any specific material. All three groups had their wounds covered with Tegaderm film (Table 1). All of the dressings were checked daily to ensure that they remained in place, and any dressings suspected of contamination were immediately replaced.

Wound biopsy and histological examination

Biopsies were performed on days 3, 7, and 21 post-wound creation, and the specimens were utilized for a histological evaluation. Four wounds were randomly selected from each rat, which were biopsied using a 3-mm-diameter biopsy punch to obtain *en bloc* tissue samples. The samples were fixed in a 10% formalin solution, embedded in paraffin, and sectioned for hematoxylin and eosin (H&E) staining. A total of 36 samples were analyzed. The slides were scanned using a microscope digital camera (DP 28; Olympus) and visualized using Olympus cellSens software (Olympus).

The histological parameters used to compare the wound-healing processes included macrophage infiltration, fibroblast

Table 1. Dressing materials used in each group

Group	Tegaderm	Connettivina gel	Duoderm gel
Control	O	X	X
Group A	O	O	X
Group B	O	X	O

Group A, using HA/silver sulfadiazine gel; Group B, using hydrocolloid gel.

infiltration and collagen deposition. A comparative analysis was performed among the groups, and the data were evaluated using a semi-quantitative scoring system based on Gupta's method, as follows [5]: 0, no infiltration; 1, mild infiltration; 2, moderate infiltration; and 3, severe infiltration. Additionally, the extent of neovascularization was assessed by quantifying the newly formed vascular structures and scoring them as follows: 0, no vascular formations; 1, a few vascular formations; 2, moderate vascular formations; and 3, severe vascular formations. For wounds that had re-epithelialized, the thinnest portion of the newly formed epithelium was measured in micrometers for comparison among the groups. The histological analysis was conducted by one histologist and one dermatologist, both of whom were thoroughly blinded to the group information.

Statistical analysis

The results obtained using the semi-quantitative scoring system were expressed as mean ± standard deviation. The Kruskal-Wallis test was performed daily for statistical evaluations. All computed values and corresponding graphs were generated using SPSS version 29.0 (IBM Corp.). Statistical significance was set at $p < 0.05$.

RESULTS

Throughout the 3-week experiment, macroscopic observations revealed by approximately day 14, all of the wounds had healed, followed by wound contraction without noticeable scarring. Additionally, no complications such as infections were observed in the wound areas, and none of the subjects showed any abnormalities in their vital signs. The data were organized into tables by day for statistical analysis (Tables 2-4).

On day 3 after wound creation, significant inflammatory cell infiltration was observed in group A compared to the other groups ($p = 0.045$). Collagen deposition, which is indicative of extracellular matrix (ECM) formation, was also higher in group A than in the other groups ($p = 0.040$). On day 7 post-wound creation, while inflammatory cell infiltration remained elevated without significant differences, angiogenesis, measured by neovascularization, showed the highest values in group A, followed by group B and the control group ($p = 0.011$). By day 21 post-wound creation, all of the wounds had fully healed, and were filled with new epithelial cells. The thickness of the new epithelial layer, indicating the maturity of the inflammation and fibroblasts, was significantly greater in group A than the other two groups ($p = 0.037$). These results were prominently observed after H&E staining (Fig. 2). As shown in Fig. 2A-C, an increase in the concentration of inflammatory cells, such as neutrophils, was observed. Fig. 2B shows a higher concentration of these cells near the epidermis compared to that shown in Fig. 2A and 2C. Additionally, although inflammatory cell infiltration remained high, increased angiogenesis was seen in the surround-

Table 2. Wound healing parameters on day 3 using a semi-quantitative scoring system

Parameter	Control	Group A	Group B	*p*-value
Inflammation	2.00 ± 0.81	2.75 ± 0.50	2.00 ± 0.50	0.045
Angiogenesis	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	-
Fibroblast	1.75 ± 0.50	1.50 ± 0.58	1.50 ± 0.58	0.730
Collagen fiber	1.50 ± 0.58	2.75 ± 0.50	1.50 ± 0.58	0.040
Re-epithelization (μm)[a]	0	0	0	0

Values are presented as mean ± standard deviation. Using a semi-quantitative scoring system: 0, absent; 1, mild; 2, moderate; 3, severe.
Group A, using HA/silver sulfadiazine gel; Group B, using hydrocolloid gel.
[a]Re-epithelization is the thinnest portion of the newly formed epithelium.

Table 3. Wound healing parameters on day 7 using a semi-quantitative scoring system

Parameter	Control	Group A	Group B	*p*-value
Inflammation	2.25 ± 0.50	2.50 ± 0.58	2.50 ± 1.00	0.671
Angiogenesis	1.25 ± 0.50	3.00 ± 0.00	1.75 ± 0.58	0.011
Fibroblast	2.25 ± 0.50	2.75 ± 0.50	2.50 ± 0.58	0.400
Collagen fiber	1.50 ± 0.58	1.75 ± 0.50	2.50 ± 0.58	0.180
Re-epithelization (μm)[a]	0	0	0	0

Values are presented as mean ± standard deviation. Using a semi-quantitative scoring system: 0, absent; 1, mild; 2, moderate; 3, severe.
Group A, using HA/silver sulfadiazine gel; Group B, using hydrocolloid gel.
[a]Re-epithelization is the thinnest portion of the newly formed epithelium.

Table 4. Wound healing parameters on day 21 using a semi-quantitative scoring system

Parameter	Control	Group A	Group B	*p*-value
Inflammation	1.00 ± 0.82	1.25 ± 0.96	0.50 ± 0.58	0.411
Angiogenesis	1.00 ± 0.00	1.75 ± 0.00	1.00 ± 0.00	0.111
Fibroblast	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	-
Collagen fiber	1.75 ± 0.50	2.25 ± 0.50	2.00 ± 0.00	0.503
Re-epithelization (μm)[a]	267.75 ± 118.88	695.00 ± 221.14	370.25 ± 62.14	0.037

Values are presented as mean ± standard deviation. Using a semi-quantitative scoring system: 0, absent; 1, mild; 2, moderate; 3, severe.
Group A, using HA/silver sulfadiazine gel; Group B, using hydrocolloid gel.
[a]Re-epithelization is the thinnest portion of the newly formed epithelium.

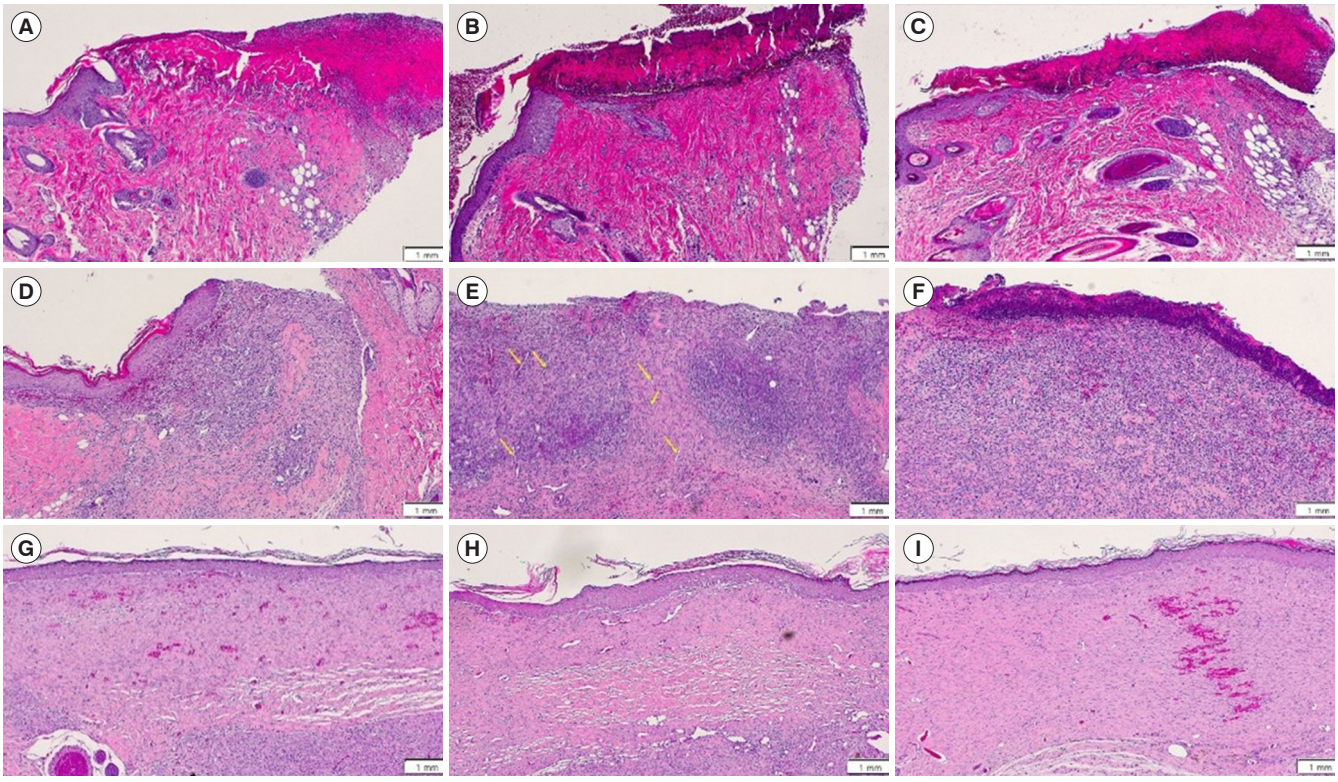

Fig. 2. Histological analysis of wound healing across different groups at various time points (hematoxylin and eosin stain, ×100). (A) Control group on day 3. (B) Group A on day 3. (C) Group B (hydrocolloid gel) on day 3. (D) Control group on day 7. (E) Group A on day 7. (F) Group B on day 7. (G) Control group on day 21. (H) Group A on day 21. (I) Group B on day 21.

ing area (compare Fig. 2E to Fig. 2D and 2F). Furthermore, group A shows a thicker re-epithelialization layer compared to the other groups (Fig. 2H).

DISCUSSION

The wound-healing process is a highly complex sequence of events involving overlapping stages: hemostasis, inflammation, proliferation, and maturation. Each stage relies on specific cytokines and growth factors for proper signaling and progression. Hemostasis begins with fibrin activation shortly after injury, forming blood clots that induce vasoconstriction and create a provisional matrix for inflammation. Neutrophils, monocytes, and fibroblasts migrate to the wound site during this phase to initiate healing. Inflammation persists for 2–3 days post-injury, with neutrophils playing a primary role as the first inflammatory cells to activate the complement system, target surrounding pathogens, and aid in tissue repair. Monocytes transform into macrophages within the wound bed to phagocytose dead cells and debris, while activating numerous cytokines and growth factors to facilitate creation of the ECM, fibroblasts, smooth muscle cells, and endothelial cells, preparing for the next phase. During the proliferation phase, growth factors secreted by macrophages, including fibroblast and vascular endothelial growth factors, stimulate fibroblast proliferation and angiogenesis, which are key to the subsequent granulation tissue formation and wound closure. Proteases, produced as collagen production increases, contribute to the formation of the new ECM, initiating the formation of granulation tissue approximately 5 days post-injury. Re-epithelialization begins concurrently, minimizing the area of the wound that requires recovery by inducing adjacent tissues to contract. Maturation involves apoptosis or the departure of the majority of the macrophages and fibroblasts from the wound site, while collagen fiber alignment enhances the tensile strength of the skin, aiding in restoring the skin to its pre-injury state. However, delayed or prolonged wound healing can lead to chronic wounds and an increased risk of scarring [6,7].

Dressings can accelerate the effective wound-healing process by aiding the activation of each phase when appropriately applied to the wound. Optimal wound dressings should adhere well to the damaged tissue, maintain proper moisture levels to prevent external infections [8], and be amenable to periodic replacement with reasonable cost efficiency. Because wound formation can vary widely in cause and presentation, the selection of suitable dressing materials is crucial. Ongoing development

offers diverse options to maintain a moist environment, such as films, foams, hydrogels, and hydrofibers [9]. In the present study, we aimed to compare the effects of two hydrogel-based products, namely HA and hydrocolloid. Hydrogels necessitate secondary dressings enveloped with film. Additionally, a control group was included with only film dressings to eliminate potential variables from this approach.

The Duoderm hydroactive gel used in group B is a hydrogel dressing composed of natural hydrocolloids, including gelatin, pectin, and sodium carboxymethylcellulose [10]. Widely used for treating various wounds, this hydrogel is valued for its non-irritating, non-sensitizing properties, and compatibility with the pH of human skin. Additionally, the gel is sometimes used with silicone foam dressing to prevent pressure damage [11]. However, as a hydrogel, Duoderm hydroactive gel lacks strong adhesion and has a limited capacity to absorb large exudates, requiring the use of secondary dressings.

In contrast to group B, the Connettivina gel used in group A is a hydrogel dressing composed of 200 kDa low molecular weight HA, a linear polysaccharide consisting of repeated units of glucuronic acid and N-acetylglucosamine disaccharide. First discovered by Meyer and Palmer in 1934, HA has since gained attention for its abundance in the human body, particularly in the dermal layers of the skin [12]. HA is known to activate components such as macrophages, fibroblasts, and collagen, thereby facilitating the inflammatory phase. Moreover, HA promotes vascular formation and regulates wound hydration and osmoregulation [13]. Consequently, it is suitable not only for wounds caused by injury but also for maintaining scalp hydration after laser treatments or hair transplant procedures, where increased hydration is beneficial. Additionally, hyaluronidase, which can break down HA, demonstrates effectiveness in treating microstomia based on this principle [14]. However, HA-based hydrogels have drawbacks such as weak mechanical properties and rapid degradation [15], necessitating caution in their use. In this study, we compared widely used hydrocolloid-based hydrogels with HA-based hydrogels to assess their effectiveness in wound healing under similar conditions.

Visual observation alone has limitations in assessing the effectiveness of the previously mentioned processes. Instead, proper histological evaluation allows for accurate and detailed assessment by measuring essential components present in each phase. Although some studies have utilized precise and detailed quantitative scoring systems to quantify these components [16], such methods may not clearly differentiate between compared values owing to the nature of the process, thereby limiting comparability [17]. In the present study, we employed a semi-quantitative scoring system to compare the distribution of cells or vessels present in each phase of healing.

The findings of this study confirmed significant differences ($p < 0.05$) in several parameters among the three groups. Specifically, inflammatory cell infiltration, peaking around day 3, was notably higher in group A than in the control group and group B. Additionally, group A exhibited greater collagen deposition, suggesting that HA facilitates signaling in the dermal layer and promotes chemotaxis of surrounding inflammatory cells to the wound site [18]. Moreover, on the 7th day post-wound, during the proliferation phase, group A showed significantly higher neovascular infiltration than the other groups, attributable to HA's known effect in promoting vascular formation [19]. Furthermore, by the 21st day, nearly all wounds showed re-epithelization, with group A demonstrating significantly higher thickness at the injury site than the other two groups, indicating accelerated tissue regeneration, potentially due to HA's effective regulation of hydration.

Despite our promising results, this study had several limitations that warrant acknowledgment. First, we experimented on the epidermis of rats rather than human skin, necessitating further research to determine whether similar significant differences would occur in human skin. Given that the wound healing mechanism in rodents occurs through the contraction of the panniculus carnosus layer, it was noted that an 8-mm wound diameter may be restrictive for comparing healing effects. Second, the sample size was relatively small, making it challenging to generalize the findings. Future studies should expand the sample size, focusing on parameters that show statistically significant differences. Third, we were unable to perform a comparison of wound size visually using gross photos, in addition to the histological comparison. Fourth, using HA containing silver sulfadiazine instead of simple HA might have introduced a degree of error into the results. Silver sulfadiazine is a sulfa-derived antibiotic medication that operates through inhibiting the growth of bacteria. Finally, this study did not investigate wound maturation or scarring beyond the 21st day of healing. Subsequent evaluation of dermal thickness via ultrasound can be helpful in understanding the patterns of scar formation thereafter [20]. Therefore, we recommend that follow-up studies be conducted to further explore these aspects of wound recovery.

Despite various experimental and comparative studies on the wound healing effects of HA being published, this paper is valuable as we objectively compared these effects with those of a similar hydrogel type dressing product, hydrocolloid, which is widely used in our hospital. Although there are limitations to generalizing these findings, we anticipate future research to broaden the range of materials available for wound treatment.

These findings highlight the potential of HA-based dressings to improve clinical outcomes in wound care, indicating opportunities for advancing therapeutic approaches.

NOTES

Conflict of interest

Young Cheon Na is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Ethical approval

The study was approved by the Institutional Animal Care and Use Committee of Wonkwang University Hospital University (IACUC No. WKU 23-82).

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