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ORIGINAL RESEARCH

EFFECTIVENESS OF SINGLE VERSUS REPEATED TOPICAL APPLICATION OF 0.05% TRETINOIN ON COLLAGEN SYNTHESIS IN ACUTE FULL-THICKNESS WOUNDS: AN EXPERIMENTAL STUDY IN RATS

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ABSTRACT

Background: Effective wound healing is essential in surgical and traumatic care, especially for full-thickness injuries. Tretinoin, a topical retinoid, has been reported to accelerate healing by stimulating fibroblast proliferation, angiogenesis, epithelialization, and collagen deposition. Its action involves modulation of TGF-β, which upregulates fibroblast-derived bFGF and promotes matrix remodeling. However, the comparative effectiveness of single versus repeated applications remains unclear.

Methods: A randomized post-test only experimental study was conducted on 27 healthy male Wistar rats (200–250 g), divided into three groups (n=9): control (A), single application of 0.05% tretinoin (B), and repeated applications for five consecutive days (C). Standardized 20 mm full-thickness dorsal wounds were created and stented with silicone rings. Group A received no treatment; Group B received a single application; Group C received daily applications for five days. On day 5, wound tissues were collected for analysis of collagen density (Masson's Trichrome) and TGF-β expression (immunohistochemistry). Data were analyzed using Shapiro-Wilk, Kruskal-Wallis, and Mann-Whitney U tests.

Results: Repeated topical application of 0.05% tretinoin (Group C) resulted in the highest collagen density and TGF-β expression among the three groups. Statistical analysis confirmed a significant difference in both parameters ($p < 0.05$). Post hoc comparisons showed that Group C differed significantly from Group A in both collagen density and TGF-β expression, while Group B (single application) did not differ significantly from either Group A or C. Histological evaluation supported these findings, revealing denser, more organized collagen fibers and stronger TGF-β immunostaining in Group C.

Conclusion: Repeated topical application of 0.05% tretinoin significantly enhances collagen synthesis and TGF-β expression in acute full-thickness wounds. This study supports the therapeutic potential of repeated tretinoin dosing in promoting faster and more robust wound healing, with implications for optimizing topical treatment regimens in clinical practice.

Keywords**:** Tretinoin; collagen synthesis; wound healing; TGF-β; full-thickness wound

INTRODUCTION

Wound healing is a central concern in plastic surgery, encompassing injuries from trauma, systemic disease, or surgical procedures. A wound is defined as a disruption of tissue continuity, and its healing progresses through three overlapping phases: inflammation, proliferation, and remodeling.[1] Several intrinsic and extrinsic factors may delay this process, potentially increasing morbidity, prolonging treatment, and leading to suboptimal aesthetic outcomes.[2] Acute wounds typically resolve within three to four weeks, whereas chronic wounds persist beyond four to six weeks and often arise from poorly managed acute injuries.[1]

Tretinoin, a topical retinoid derived from vitamin A, has demonstrated benefits in enhancing various aspects

of wound healing, including angiogenesis, fibroblast proliferation, epithelialization, and collagen synthesis.[3] Clinically, it has been used to prevent post-dermabrasion hyperpigmentation and to manage chronic wounds such as pressure ulcers and diabetic ulcers.[4] In 2001, Paquette et al. reported that topical tretinoin application to full-thickness wounds stimulated granulation tissue formation, neovascularization, and collagen deposition.[5] Toyama et al. later evaluated its effectiveness in rats using short-contact application (five minutes daily) versus no treatment, and found enhanced healing in the treated group.[6] Prolonged application under transparent dressings may induce irritation and delay healing due to persistent inflammation, but findings remain inconclusive..[5]

Other studies suggested that topical retinoids may counteract steroid-impaired healing by restoring transforming growth factor-beta (TGF-β) and insulin-like growth factor 1 (IGF-1) levels, thereby reactivating collagen production.[7] This study aimed to compared single and repeated 0.05% tretinoin applications on collagen synthesis and TGF-β expression during full-thickness wound healing in rats.

METHODS

Animal Model and Experimental Design

This was a randomized post-test-only experimental study conducted on 27 healthy male Wistar rats (Rattus norvegicus) aged 3–4 months and weighing 200–250 grams. Animals were obtained from a certified breeder and housed in ventilated polypropylene cages lined with husk bedding, four rats per cage, at a controlled room temperature of 32°C. All rats received standard pellet feed (PAR-G, 20 g/day) and water ad libitum. Ethical approval for all procedures was granted by the Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine, Universitas Airlangga (No. 2.KEH.22.02.2025). The rats were randomly assigned into three groups (n = 9 per group): Group A (control), Group B (single application of 0.05% tretinoin), and Group C (repeated application for four consecutive days). All wounds were created and evaluated on day 5 of the experiment. Study design and experimental workflow are shown in Figure 1.

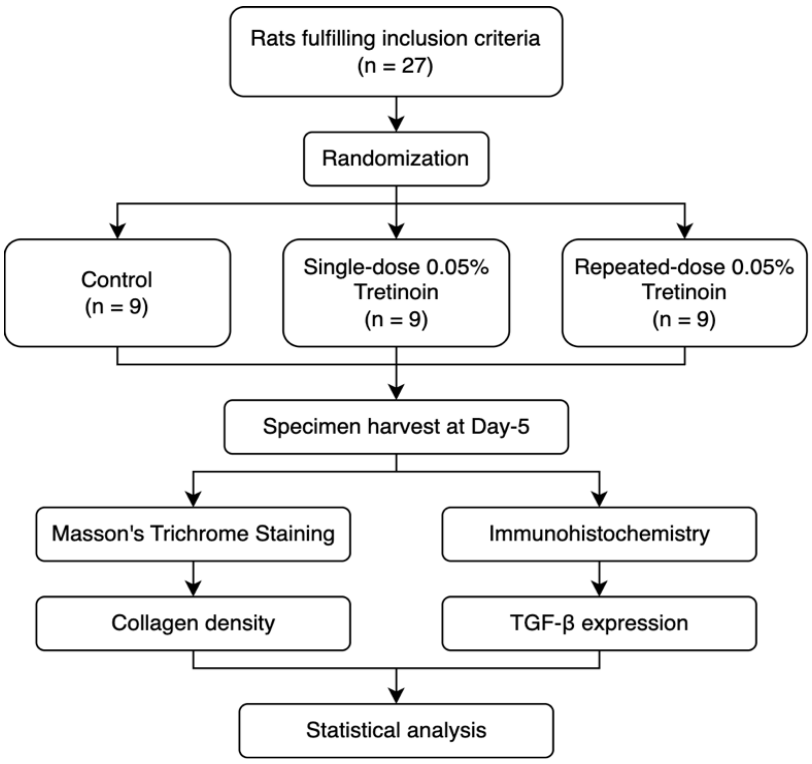

Figure 1. Study design and experimental workflow

Wound Creation and Tretinoin Application

Each rat underwent general anesthesia with intramuscular ketamine (10 mg/kg). The dorsal skin was shaved, disinfected with 10% povidone-iodine and Savlon (1:30), and isolated with sterile drapes. A full-thickness circular excision measuring 20 mm in diameter was made using a scalpel, and a silicone stent was sutured to the wound edge using nylon 5.0 to prevent wound contraction. In the control group, no topical agent was applied. Group B received a single application of 0.05% tretinoin solution topically for 5 minutes immediately after wounding, followed by rinsing

with 0.9% NaCl and application of a transparent dressing. Group C received the same procedure daily for four consecutive days. All animals were housed in larger individual cages (50×70 cm) until sacrifice.

Tissue Collection and Outcome Evaluation

On day 5, all rats were euthanized via intraperitoneal phenobarbital injection (60–200 mg/kg). Skin specimens including subdermal tissue were excised and fixed in 10% buffered formalin. Tissue sections (4–6 µm) were stained with Masson's Trichrome and observed under a light microscope at 400× magnification using an AccuScope device. Collagen density was scored semi-quantitatively from 0 (no collagen fibers) to 4 (very dense fibers) based on histopathological evaluation.[8] For TGF-β expression, immunohistochemical analysis was performed using anti–TGF-β primary antibody (1:200). Sections were incubated at room temperature for 90 minutes or overnight at 4°C, with antigen retrieval in citrate buffer. Staining was visualized under a light microscope, and expression was quantified as the percentage of TGF-β–positive cells per 100 high-power fields using AccuView software.

Statistical Analysis

All data were analyzed using SPSS version 26 (IBM Corp.). The Shapiro–Wilk test was applied to assess data normality. For non-normally distributed variables, the Kruskal–Wallis test was used, followed by Mann–Whitney U test for pairwise comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS

Collagen Density

Collagen density was evaluated on day 5 using Masson's Trichrome staining at 400x magnification (Figure 2). The control group (Group A) showed sparse collagen fibers, whereas both tretinoin-treated groups demonstrated more pronounced collagen presence. Group C, which received repeated topical applications of 0.05% tretinoin, showed the densest collagen network.

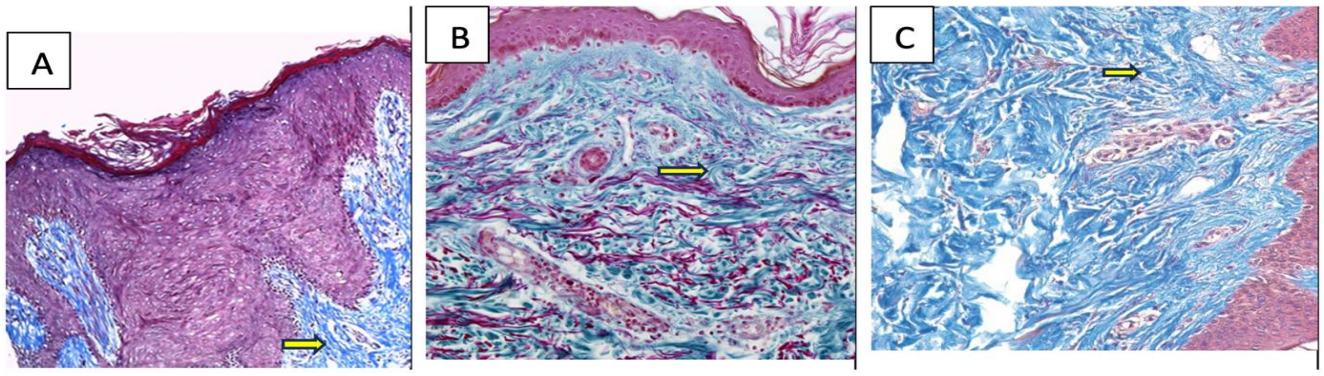

Figure 2. Collagen density evaluation using Masson's Trichrome staining on day 5 at 400× magnification. A. Control; B. Single application of 0.05% Tretinoin; C. Repeated application of 0.05% Tretinoin (five times).

Descriptive analysis (Table 1) revealed that the mean collagen density score in the control group was 2.11 ± 0.33. Group B, which received a single application, had a higher mean score of 2.56 ± 0.88, while Group C showed the highest mean at 3.22 ± 0.66. Boxplot distribution (Figure 3) illustrated a clear upward trend in collagen density from control to repeated application.

Table 1. Descriptive data and Kruskal-Wallis analysis of collagen density based on groups

Group	Sample size, n	Median (Min-Max)	Mean ± SD	p-value
Control	9	2.0 (2.0-3.0)	2.11 ± 0.33	0.007*
Single-dose tretinoin	9	3.0 (1.0-4.0)	2.56 ± 0.88	
Repeated-dose tretinoin	9	3.0 (2.0-4.0)	3.22 ± 0.66	

*p-value significant at <0.05; SD: Standard deviation.

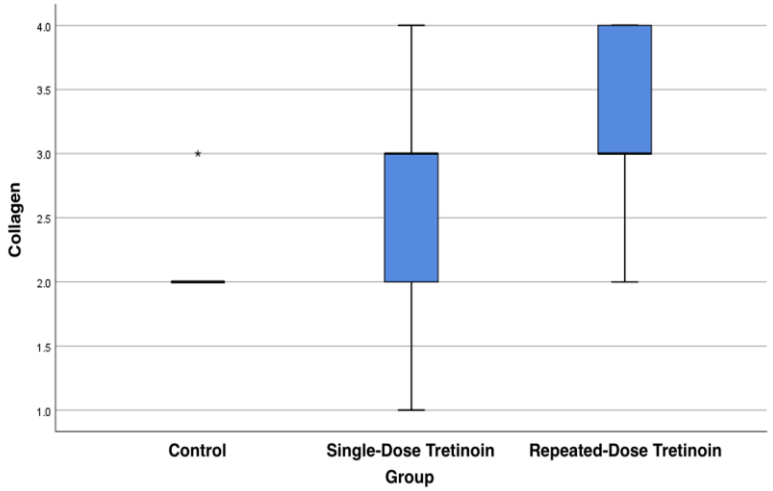

Figure 3. Box plot showing data distribution in Groups I (Control), II (Single Tretinoin), and III (Repeated Tretinoin).

Given the ordinal nature of the collagen scoring system, a Kruskal-Wallis test was performed. The test yielded a significant difference among the three groups ($p < 0.05$; Table 1), indicating that at least one treatment group differed from the others. A subsequent Mann-Whitney post hoc analysis confirmed a statistically significant difference between Group A and Group C ($p = 0.020$), but no significant difference between Group A and Group B ($p = 0.133$) or between Groups B and C ($p = 0.382$).

TGF-β Expression

TGF-β expression was analyzed via immunohistochemistry on day 5, visualized under a light microscope. Representative histological images (Figure 4) showed the weakest staining in Group A, with more intense TGF-β expression observed in Group B and strongest in Group C.

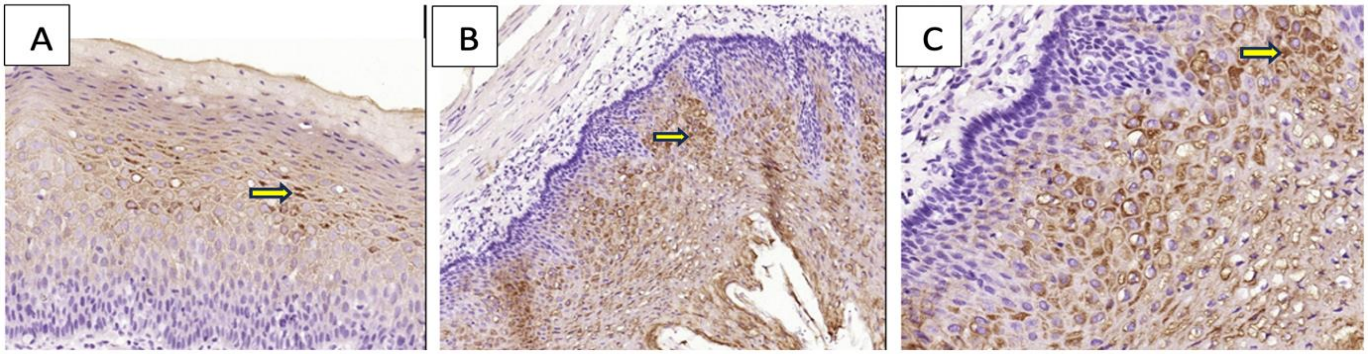

Figure 4. Immunohistochemical evaluation of TGF-β expression on day 5 at 400× magnification. A. Control; B. Single application of 0.05% Tretinoin; C. Repeated application of 0.05% Tretinoin (five times).

Quantitative analysis using AccuView software revealed mean TGF-β expression levels of 8.89% ± 3.03 in Group A, 18.89% ± 4.59 in Group B, and 33.33% ± 5.64 in Group C (Table 2). Statistical analysis showed that the data for Group C were not normally distributed ($p = 0.015$), and the Kruskal-Wallis test was again used. The results indicated a statistically significant difference among all three groups ($p = 0.000$). Post hoc Mann-Whitney analysis revealed significant differences across all pairwise comparisons: A vs. B ($p = 0.002$), A vs. C ($p = 0.000$), and B vs. C ($p = 0.001$). These findings suggest that repeated topical application of 0.05% tretinoin significantly enhances both collagen synthesis and TGF-β expression in acute full-thickness wounds, compared to a single application or no treatment.

Table 2. Descriptive data and Kruskal-Wallis analysis of TGF- β expression based on groups

Group	Sample size, n	Median (Min-Max)	Mean ± SD	p-value
Control	9	10.68 (6.34-22.38)	12.15 ± 4.83	<0.001*
Single-dose tretinoin	9	24.30 (16.14-48.05)	28.65 ± 9.85	
Repeated-dose tretinoin	9	59.98 (54.95-71.98)	61.32 ± 6.31	

*p-value significant at <0.05; SD: Standard deviation.

DISCUSSION

Wound healing is a complex biological process involving multiple overlapping phases: hemostasis, inflammation, proliferation, reepithelialization, contraction, and remodeling. In dermal wounds, the success of this process relies heavily on fibroblast activity in synthesizing extracellular matrix components, particularly collagen. Collagen types I and III account for more than 90% of total collagen in the skin and are essential for mechanical strength and providing a scaffold for cellular adhesion and migration. The synthesis of new collagen is largely dependent on fibroblast activation, which is strongly influenced by growth factors such as transforming growth factor-beta (TGF-β). TGF-β promotes collagen gene expression, regulates fibroblast differentiation into myofibroblasts, and increases the production of other extracellular matrix proteins.[9]

Topical application of tretinoin has been shown to improve healing in diabetic foot ulcers by stimulating granulation tissue formation, collagen and fibronectin synthesis, and accelerating reepithelialization and wound contraction. Tretinoin promotes keratinocyte proliferation and differentiation, increases epidermal thickness, and facilitates superficial angiogenesis, enhancing the delivery of oxygen and growth factors to the wound site. It also stimulates mucopolysaccharide and structural protein synthesis, thereby supporting the proliferative phase of wound healing.[10]

In the present study, repeated application of 0.05% topical tretinoin significantly enhanced collagen synthesis in full-thickness wounds, as evidenced by increased collagen density on day 5. Group C, which received repeated applications, had the highest mean collagen score (3.22 ± 0.67), compared to Group B with a single application (2.56 ± 0.88) and the untreated control (2.11 ± 0.33). These results suggest that repeated administration is more effective in stimulating fibroblast activity and forming new collagen matrix during the proliferative phase. Collagen synthesis begins during the proliferative phase, typically within the first two weeks following injury, and peaks by the third to fourth week. Fibroblasts produce type III collagen early in this phase, facilitating wound closure. TGF-β drives fibroblast proliferation and the secretion of basic fibroblast growth factor (bFGF), further promoting collagen production.

Tretinoin's mechanism of action in promoting collagen synthesis involves enhancing epidermal cell proliferation and differentiation while simultaneously inhibiting matrix metalloproteinases (MMPs), which degrade the extracellular matrix.[11] Additionally, tretinoin induces mannose receptor C2 (MRC2) and prolidase expression, both of which are involved in type I collagen recycling, thereby reinforcing connective tissue during wound healing.[12] Kang et al. also reported increased immunohistochemical expression of procollagen following tretinoin treatment, consistent with this study's findings of higher collagen density and more organized fiber arrangement after repeated application.[13]

This study showed a significant increase in TGF-β expression across groups, highest in the repeated-treatment group (Group C). The Kruskal-Wallis test indicated a highly significant difference, and post hoc Mann-Whitney tests showed significant differences between all group pairs. These findings suggest that both single and repeated tretinoin applications upregulate TGF-β, with repeated application being more effective.

Previous studies have similarly reported that retinoic acid increases TGF-β expression. Kim et al. found that topical retinoic acid significantly enhanced TGF-β1 expression and, to a lesser extent, TGF-β2 in murine epidermis, as demonstrated via immunohistochemistry.[14] Wicke et al. further showed that oral all-trans retinoic acid increased collagen deposition and TGF-β levels, reinforcing the role of retinoic acid in growth factor regulation and tissue regeneration.[15]

TGF-β is known to be involved throughout all stages of wound healing. Its signaling pathway is critical for maintaining skin homeostasis and coordinating tissue regeneration after injury. While TGF-β1 suppresses keratinocyte proliferation, it simultaneously stimulates dermal fibroblast proliferation, underscoring its role in balancing tissue formation and differentiation.[16] It also regulates fibroblast chemotaxis, differentiation into myofibroblasts, extracellular matrix deposition, angiogenesis, wound contraction, and stimulation of other growth factors and MMPs.[17]

Taken together, the results of this study demonstrate that repeated topical application of 0.05% tretinoin significantly increases collagen density and TGF-β expression in full-thickness wounds. These findings suggest that tretinoin may serve as a valuable adjuvant therapy for enhancing the proliferative phase and improving overall wound healing outcomes. Clinically, repeated topical tretinoin application could be considered as an adjunct treatment in acute wound management, especially in cases requiring accelerated tissue regeneration and optimized collagen synthesis.

CONCLUSION

Repeated topical application of 0.05% tretinoin significantly increased collagen density and TGF-β expression compared to single application in full-thickness wound healing. These findings highlight tretinoin's potential to enhance the proliferative phase and overall tissue regeneration, warranting further studies to optimize dosing strategies, treatment intervals, and translational potential to human models.

DECLARATIONS

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Competing and conflicting interests

The authors declare no competing interests or conflicts of interest related to this work.

Ethical approval

Ethical approval for all procedures was granted by the Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine, Universitas Airlangga (No. 2.KEH.22.02.2025), confirming adherence to ethical standards in the treatment of animal subjects.

Informed consent

Not applicable.

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