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# Comparison of the effectiveness between single and repeated administration of topical Tretinoin 0.05% on full-thickness acute wound healing

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## ABSTRACT

**Background:** A wound is a discontinuity of any bodily tissue due to various causes. Every wound of any severity could result in problems related to its healing process, such as scar tissue formation. According to the duration of its healing process, wound is divided into 2 categories: acute wound that takes approximately 3-4 weeks to heal and chronic wound that needs 4-6 weeks and resulted from inappropriate healing of the acute wound. Tretinoin's use in wound healing has been controversial for more than 40 years. Topical Tretinoin has keratolytic effect that stimulates fibroplasia and epithelization on full-thickness wounds. On the other hand, single administration along with long-contact topical Tretinoin has an irritative effect that could delay healing due to continuous inflammation. This study aims to explore the effectiveness of single administration compared to repeated administration of topical Tretinoin in full-thickness acute wound healing on rats at Faculty of Veterinary Medicine, Airlangga University, Surabaya.

**Methods:** This experimental study involved 27 male rats which were randomly divided into 3 groups: control (A), single-administration (B), and repeated-administration (C). Full-thickness wound size 20 mm in diameter was made on the skin of each rat. On group A, wounds were covered with Tulle and transparent dressing. On group B, wounds were treated with lidocaine 2% and tretinoin solution 0,05% in single administration, while the same treatment was given repeatedly on group C for 4 days straight. Evaluation was done microscopically on day 5 according to epithelization phase on wound healing with hematoxylin and eosin (H&E) staining.

**Results:** The result from the calculation showed that group C obtained a significantly higher average number of fibroblast cells count compared to the treatment group A and B. The average number of fibroblast cells in treatment group B was three times higher than the number of fibroblast cells in treatment group A, while the average number of fibroblast cells in treatment group C was three times the number of fibroblast cells in treatment group B. From the statistic, it was proven that the regular application of 0.05% topical tretinoin solution in a full-thickness wound (Group C) has shown a significant increase in the number of fibroblasts during the proliferative phase.

**Conclusion:** A frequent administration of 0.05% topical tretinoin solution resulted in a higher number of fibroplasia improvements compared to once administered in full thickness acute wound healing, however it did not result in epithelialization on day 5.

**Keywords:** Tretinoin, retinoic acid, fibroblast growth factor, epithelialization.
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## INTRODUCTION

The wound is a discontinuity of bodily tissue due to various causes, such as trauma, thermal changes, chemical exposure, explosion, electric shock, and animal bite. Every wound of any severity could result in problems related to its healing process, including pain and delayed healing which could increase cost of treatment and limited activity. Incorrect management of skin wounds may result in the formation of scar tissue. The wound itself is divided into 2 categories based on the duration of its healing process: acute wound that takes approximately 3-4 weeks to heal and chronic wound that resulted from acute wound and needs 4-6 weeks to heal.[1]

Tretinoin's use in wound healing has been controversial since more than 40 years ago.[2] Up until now, Tretinoin has been used as topical treatment to promote healing and prevent hyperpigmentation on dermal abrasion due to carbon dioxide laser or chemical peeling. It is also used as a secondary dressing on chronic wounds such as decubitus and diabetic ulcers that could not undergo surgical treatment.[3] Tretinoin acts as modulator to increase production of basic fibroblast growth factor (bFGF) which stimulates angiogenesis.[4-6]

Topical Tretinoin has keratolytic

effect. A study by Paquette et al.[5] stated that topical Tretinoin could stimulate FGF-2 production to increase fibroplasia, angiogenesis, and granulation, promoting epithelization on full-thickness wounds. Study on rats by Toyama et al.[7] also found that administration of topical Tretinoin for 5 consecutive days with short-contact (5 minutes) method, followed by rinsing using normal saline to reduce irritation, resulted in significantly quickened epithelization compared to control which only used transparent dressing. On the other hand, single-administration of topical Tretinoin with long-contact method had irritative effects that might delay healing due to continuous inflammation, although there is no definitive proof yet.[5]

## METHOD

This experimental study involved 27 male Wistar rats (*Rattus novergicus*) which were randomly divided into 3 groups: control (A), single-administration of Tretinoin (B), and repeated-administration (C); each group consisted of 9 samples.

Wounds were made on the skin of the rats in sterile environment after anesthesia. Wounds were made using mesh with a diameter of 20 mm as deep as full thickness.

Tulle and transparent dressing only covered wounds on group A. On group B, wounds were treated by lidocaine 2% and tretinoin solution 0.05% for 5 minutes, then rinsed by NaCl 0.9% before Tulle and transparent dressing covered them to prevent contamination. Similarly, group C was also given the same treatment as group B. However, the treatment on group C was repeated for 4 consecutive days.

Assessments were done on the fifth day after the start of treatment, referring to the predicted epithelization of wound healing. Rats were euthanized using phenobarbital 60-200 mg/kgBW intraperitoneal. The areas of healed wounds were measured using visitrak to evaluate epithelization. Then, skin and tissue as deep as sub-dermal fat was taken to be examined histopathologically using microscope.

Haematoxylin and eosin (H&E) staining were done on each specimen. The specimens were observed using microscope with 400 times magnification for 5 different fields of view. Fibroblasts were counted using software cell count to determine the expression of healing pattern.

Data obtained were analyzed. Comparisons were analyzed using Kruskal Wallis and Mann-Whitney test using non-parametric data.

## RESULT

Experiments were carried out to determine the impact of treatment. Without Tretinoin (A), Tretinoin solutio with a concentration of 0.05% and Lidocaine 2% given topically together with the one-time method (B) and Tretinoin solutio with a concentration of 0.05% and Lidocaine 2% which is given topically by the method of repeated administration (C) to epithelial cells and fibroblasts. The experimental animals, Rattus novergicus strain wistar, were divided into 3 groups. There are 9 mice in each group. The body weight and age of the mice were confirmed to be homogeneous and were confirmed not factors that affected fibroblast and epithelial cells.

The treatment of each study group was carried out according to the procedure described before. The use of a silicone stent on the full-thickness wound margin as shown in Figure 1 and was maintained until day 5. At the end of the study, the wound was excised until the surrounding tissue was as deep as the fascia as shown in Figure 2.

After day 5, the granulation tissue in group C was thicker than that in group B, while the granulation tissue in group B was thicker than in group A. In all groups, there was no epithelial growth at the wound edges (Figure 3).

A significant difference was obtained by microscope examination. Group C found thicker granulation tissue and more new vascular tissue than group B. Group A had the thinnest granulation tissue thickness and the least amount of vascularity of the three treatment groups.

**Figure 1.** Use of a silicone stent on a full-thickness wound edge to avoid epithelialization bias which can be confused with rat skin retraction (stitched using Nylon 5.0 thread).

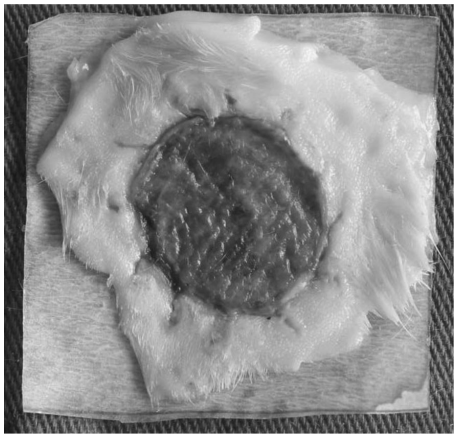

**Figure 2.** Skin tissue from full-thickness wound excision on the back of mice taken on day 5 (after removing the silicone stent).

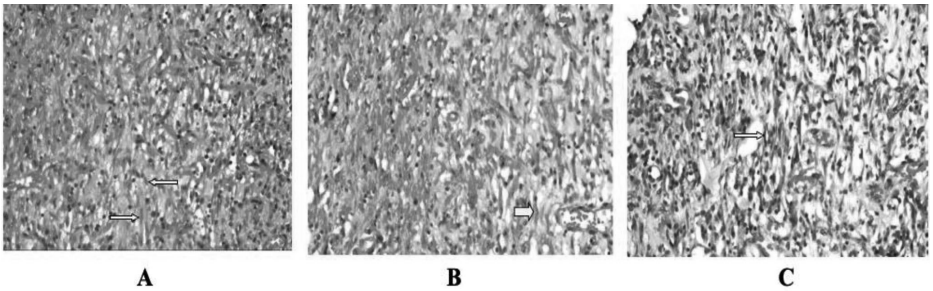


**Figure 3.** Microscopic image with 400x magnification showing the comparison of fibroblast cells in treatment group A (figure A), treatment group B (figure A), and treatment group C (figure C).

The number of fibroblast cells as the variable studied was counted using a microscope with 400x magnification in 10 fields of view.

### Fibroblast Count Result

The result shows that the average fibroblast cell in group C, the group that received Tretinoin solution with a concentration of 0.05% and Lidocaine 2% which was given topically with repeated administration method was higher, which is 762.33 + 90.330. Group B received Tretinoin solution with a concentration of 0.05% and Lidocaine 2% which was given topically with a one-time lower method of 397.56 + 77.135. The lowest was in group A, without treatment, resulting in 111.33 + 39.494 (Table 1).

**Table 1. Description of fibroblast cells.**

| Group | N | Mean (cell) | Std. Deviation |
|---|---|---|---|
| A | 9 | 111.33 | 39.494 |
| B | 9 | 397.56 | 77.135 |
| C | 9 | 762.33 | 90.330 |

**Table 2. Anova test results on fibroblast.**

| Group | Mean | Std. Deviation | F Test | p-value |
|---|---|---|---|---|
| A | 111.33 | 39.494 | 183.454 | 0.000 |
| B | 397.56 | 77.135 | | |
| C | 762.33 | 90.330 | | |

**Table 3. Tukey HSD test results on fibroblast cells.**

| Group | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| A | 9 | 111.33 | | |
| B | 9 | | 397.56 | |
| C | 9 | | | 762.33 |

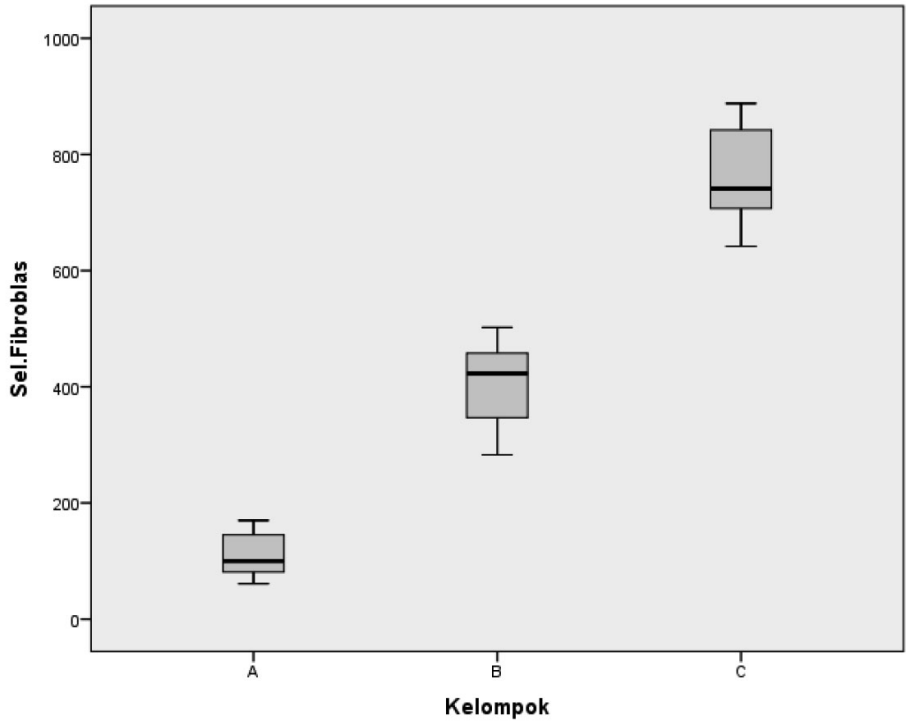


**Figure 4.** Distribution of data with box plots in groups A, B, and C.

In Figure 4 it can be seen that the distribution of data in group C is higher than in group B and the distribution of data in group A is low. The center line in the figure shows the median value. The median value in the three groups is not exactly in the middle of the box plot.

In Figure 5 it can be seen that the average of group C is the highest compared to groups B and A. These results support the narrative that the administration of Tretinoin solution with a concentration of 0.05% and Lidocaine 2% given topically with the repeated administration method produces the most fibroblast.

The ANOVA test was used to compare the number of treatments with more than 2 groups, and the tested data passed the normality test (the data were declared normal). The results of the ANOVA test were declared significant if the p-value was 0.05. Based on the results of the ANOVA test, a significance value of 0.000 was obtained where the value was less than 0.05. So it can be concluded that there are differences in the number of fibroblast cells between the 3 groups A, B, and C (Table 2). Because there are differences between groups, it is necessary to test to determine which group pairs are different. Further or post hoc tests with multiple comparisons are carried out. The post hoc test used the Tukey Honestly Significant Difference (HSD). It is said that there is a difference if each group is in a different column.

Table 3 shows that groups A, B, and C are in different columns, thus fibroblast cells in group A are significantly different from groups B and C and fibroblast cells in group B are significantly different from group C.

## DISCUSSION

The evaluation was carried out on day 5 by performing an excision at 1 cm from the edge of the skin tissue around the wound as deep as the fascia. Visually, it was seen that the granulation tissue in Group C was thicker than in Group B and the granulation tissue in Group B was thicker than in Group A, but there was no epithelialization at the wound edges in all study samples.

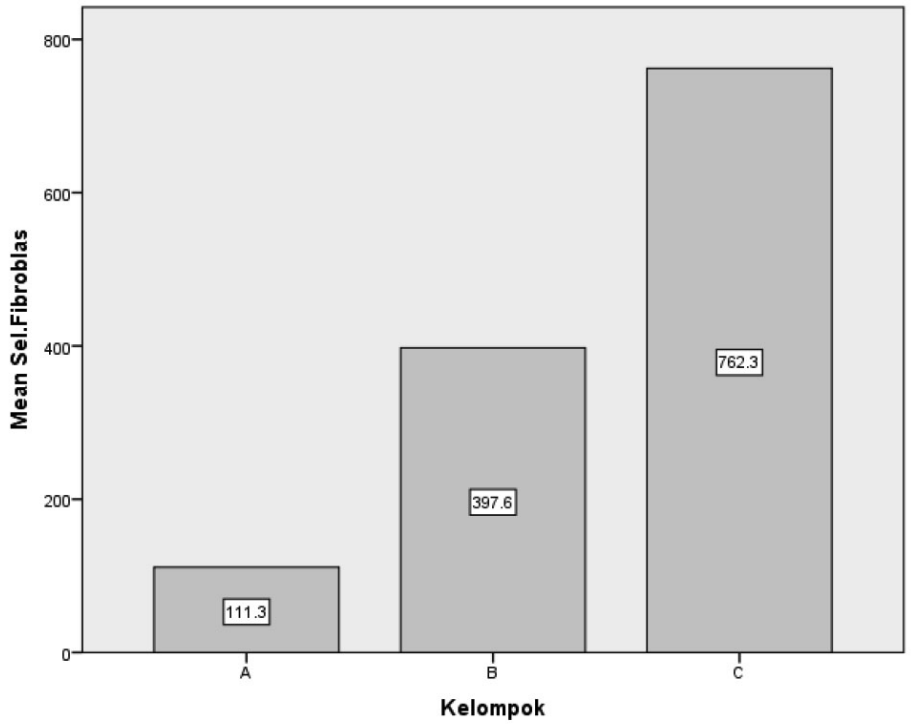


**Figure 5.** Comparison of mean values in groups A, B and C.

In the same study conducted by Matsumoto et al.[6] the use of silicone stents on the edges of full-thickness wounds to avoid epithelialization bias which can be seen more quickly due to contraction of muscle tissue (panniculus cavernous) in the skin of rodents (including rats) which allows contraction to occur. to help accelerate the closure of the defect along with the epithelialization process. In contrast to what was done by Toyama et al.[8] where a silicone stent was not placed on the edge of a full-thickness wound

Wound healing can occur from the edges and bottom of the wound by the formation of a fibrin clot which is replaced by granulation tissue and epithelial migration. An open defect will undergo a healing process with a combination of contraction, reepithelialization, and dermal reconstitution. In the full thickness model, all dermis components are involved, thus epithelialization occurs only from the wound edges.[8]

In 2001 Paquette et al.[5] stated that applying the topical Tretinoin in a full-thickness wound can increase the production of FGF-2 which results in the increase of fibroplasia, angiogenesis and granulation which eventually speed up the epithelization process. This research has come up with the same result derived from the same idea. The difference in the thickness of the tissue in histopathologic findings can be seen in the figure 3. the figure shows the granulation tissue in the treatment C (frequent application of Tretinoin topical) is thicker compared to the one in the treatment group B and group A.

The result shows that the group in treatment C has significantly higher number in. fibroblast cells count compared to the treatment in group B and group A. the average count of fibroblasts in group B is three times higher compared to the average number in group A, while the fibroblast in group C is three times the number in group B treatment.

Statically, it was proven that the frequent application of 0.05% topical tretinoin solution in full-thickness wounds (Group C) significantly increases fibroblast cells production during the proliferation phase.

A number of factors determined the secondary wound closure in the mice. In some animals with loose skin such as mice, *panniculus carnous* that was attached to the base of the dermis can help to speed up the wound healing. The open wound can go through the process of wound healing through the combination of contraction, epithelization and dermal reconstruction. In the full-thickness wound model, the epidermis layer was removed, so the wound healing only happens in the dermis and the epithelization can occur in the peri-wound area. In the mice, the acute inflammation phase only occurs in the first 3 days, it is faster compared to the inflammation phase in humans. The peak of the proliferation phase in mice occurs in the 5$^{th}$ and 6$^{th}$ day, while it occurs in the 7$^{th}$ days in human's phase. This is why, that in the 5$^{th}$ day we cannot see the epithelization in the outskirt of the wound.[8]

However this result was opposed in the studies conducted by Toyama et al.[7] with short-contact method, this method used a 5 minutes contact of the topical Tretinoin, and they would wash it out using normal saline, however they did not use a silicone stent to prevent the retraction of the skin. The result from this study showed that the wound's surface area was smaller due to the retraction of the peri-wound in the skin of the mice.

## CONCLUSION

Repeated administration of 0.05% solution of topical Tretinoin resulted in a higher increase in fibroplasia than once for full thickness acute wound healing. Repeated administration of 0.05% solution of topical Tretinoin did not result in epithelialization on day 5, either on repeated administration or once for full thickness acute wound healing.

## ETHICAL STATEMENT

This study has been approved by Ethical Committee Faculty of Medicine Universitas Airlangga/Soetomo Hospital Surabaya, Indonesia.

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None.

## CONFLICT OF INTEREST

None.

## AUTHOR CONTRIBUTION

All authors had contributed in manuscript writing and agreed for the final version of manuscript for publication.

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