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Liquid Smoke and Hydrogel Membrane as Burn Wound Healing Properties

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ABSTRACT

Background: Any person has the potential to get injured, especially burns. Hydrogel membrane and liquid smoke are a combination of antibacterial, anti-inflammatory and antioxidant properties. These properties involve an important role in wound healing. In wound healing, the re-epithelialization process is a critical parameter for the success of wound closure
Objective:. Analyzed the effect of re-epithelialization length on burns of rats given a liquid smoke combination hydrogel membrane.
Methods: Experimental research used post test only with control group design. The number of samples was 15 white wistar rats with burns which were divided into two treatment groups, namely P1 (liquid smoke combination hydrogel membrane), P2 (liquid smoke) and one control group K (hydrogel membrane). Assessment of reepithelialization length has been calculated using a microscope. Statistical test using One Way ANOVA – Post Hoc Bonferroni test.
Results: The average length of re-epithelialization (μm) for each group was (P1) 2004.91, (P2) 1534.15 and (K) 1248.16. Based on the results of the One Way ANOVA analysis, we found p value = 0.028 ($p < 0.05$). This shows that there were significant differences in the data between groups.
Conclusion: There was a significant difference on the length of re-epithelialization in rats burn wound between hydrogel membrane group with liquid smoke, liquid smoke group and control group

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1. Introduction

Annually, the incidence of burns is estimated to occur around 2.4 million cases in the world with 650,000 immediate care and 75,000 hospitalizations. 8000-12,000 cases are reported to have died due to complications from burns.[1] In 2017, WHO estimated that 180,000 people died from burns.[2] 2015 data, during 2012-2014 there were 3,518 cases of burns in several major Indonesian hospitals.[3] Epidemiological research at Cipto Mangunkusumo Hospital (RSCM) in 2011-2012 there were 303 patients with burns with a mortality rate of 34% in 2012 and 33% in 2011.[4] The majority of burns are second-degree burns, which is 73%, while first-degree burns are 17% and third-degree burns are 10%.[5]

Burns are different from other wounds. The hallmark of a burn that distinguishes it from other wounds is the generalized increase in capillary permeability leading to plasma leakage.[6] First-degree burns affect only the outer epidermis and appear reddish on the outside. Grade II includes burns of the entire epidermis and part of the dermis. The wound may be accompanied by blisters and severe pain. Grade III involves all layers of the skin. The skin will appear dry with very slow wound healing.[7]

In burns, the healing process proceeds in a similar way to other wounds. The wound healing process is a complex process consisting of various phases that are coherent and each phase can go hand in hand. The wound healing process has 3 phases, namely the hemostasis/inflammatory phase, the proliferative phase, and the remodeling phase.[8] In the proliferative phase are important processes become critical success indicators, namely wound closure reepitelisasi.[9] Reepithelialization was assessed by measuring the length of the new epithelial cells that grew microscopically with hematoxylin-eosin staining.[10] One of the proinflammatory cytokines that have a lot of influence in the reepithelialization process is TNF-α. After injury, Keratinocytes, epithelial stem cells, immediately release proinflammatory cytokines. TNF-, as a proinflammatory cytokine released by

keratinocytes, activates keratinocytes, stimulates keratinocyte migration and activates fibroblasts.[11]

Liquid smoke or liquid smoke is a liquid that contains phenol. The previous benefit of liquid smoke was as a preservative, especially in smoked fish products.[12] However, recently several studies on the benefits of liquid smoke in the medical field have been carried out by scientists from Indonesia. Previous research has proven the benefits of liquid smoke as a burn therapy in rats by applying liquid smoke directly to rats. The use of liquid smoke on the burn turned out to have a better effect than 10% povidone iodine.[13] So far, recent research on liquid smoke can increase the expression of TNF-α, a proinflammatory cytokine, which has many effects on the reepithelialization process. The effect of liquid smoke on TNF-α expression was 72.4%. The effect is stronger than BHCL which is only 33.2%.[14]

Hydrogel membranes have previously been made and tested on wounds by Indonesian researchers. Previous research has succeeded in making hydrogels with PVP and carrageenan materials. The study succeeded in proving that hydrogel was able to provide a faster healing effect on burns than the control group with sterile gauze.[15] The latest research is the study of membrane-based PVA hydrogels, chitosan and starch with a combination of liquid smoke and vitamin K. The hydrogel membranes are able to accelerate the healing of the incision in rats. Wound healing by hydrogel membrane was faster than the control group.[16] Based on the description above, it is interesting to do further research on liquid smoke and hydrogel membrane. Hydrogel membrane combined with liquid smoke will be tested on rat burns to assess the length of re-epithelialization which is one of the important histopathological indicators that need to be assessed in wound healing.

2. Methods

Material

two main material are premium grade liquid smoke and hydrogel membrane raw material. Hydrogel membrane raw material consist of PVA, chitosan, starch and acetic acid.

Subject

This experimental research was set on 15 male wistar rats with ages 3 months and weight 300 grams which were divided into 3 groups. The rats would be acclimatized in the laboratory for one week in group cages.

Preparation of Hydrogel Membranes

Chitosan 3 gr was mixed with 1% acetic acid solution to produce 3% chitosan solution, 5% starch concentration. Each solutions were mixed with 400 rpm and 70C magnetic stirrer separately. All homogenous raw materials solution were mixed and added with premium grade liquid smoke. Hydrogel membran solution formula ratio was Chitosan : Starch : PVA : Premium Liquid Smoke (6:6:4:3) ml. Hydrogel membrane solution was poured on a petri dish and roasted at 60ºC for 6 hours until the solution changed into gel. Each hyrogel membrane petri dish were cut into square shape and combined with non-woven plasters according to burn wound shape and number of sample.

Burn Wound Induction

Local anesthesia was carried out using 0.2 mL ketamine xylazine injected intraperitoneally. A 2-cm diameter iron plate was heated at 210 oC and then inducted for 2 seconds on rat's shavedback skin to make a second-degree burn wound.

Treatment

Rats were randomly assigned into three groups: (i) treatment 1 group (P1), in which rats were treated with hydrogel membrane with premium liquid smoke, once daily; (ii) treatment 2 group (P2), in which rats were treated with topical premium grade liquid smoke, once daily; (iii) 1% control group (K), in which rats were treated with membrane hydrogel, once daily.

Histopathological Examination

On the 7th day, all rats were terminated using ketamine xylazine 0.3 ml intraperitoneally and the skin tissue was taken. Histological preparations were made with hematoxylin-eosin staining to assess the length of re-epithelialization. Length was calculated in millimeters (mm). Newly growing epithelial cells were assessed from epithelial areas without hair follicles.

Statistical Analysis

Data analysis using One Way ANOVA – Post Hoc Bonferroni test.

3. Results

Table 1. Characteristics of re-epithelialization length.

Group	N	Mean (μm)	Deviation Standart	p Value
P1	5	2004.91	366.95	
P2	5	1534.15	387.02	0.028*
K	5	1248.16	402.32	

Table 2. Multiple comparisons with Post Hoc Bonferroni test.

Group	P1	P2	K
P1		0.233	0.027*
P2	0.233		0.791
K	0.027*	0.791	

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Table 1 shows the results of mean and deviations standart from length of re-epithelialization and Table 2 shows multiple comparison between two groups with Bonferroni post hoc test. Based on the results of the study, It is known that the order of the average length of re-epithelialization from the longest to the lowest is P1, P2 and K. According to analysis results from One Way ANOVA method, a significance result was obtained with p value = 0.028 (p <0.05). This result shows that there are significant differences in the data between groups. Based on the results of the Bonferroni Post Hoc test, it was found that the average comparison was significant only between groups P1 and K with p value = 0.027.

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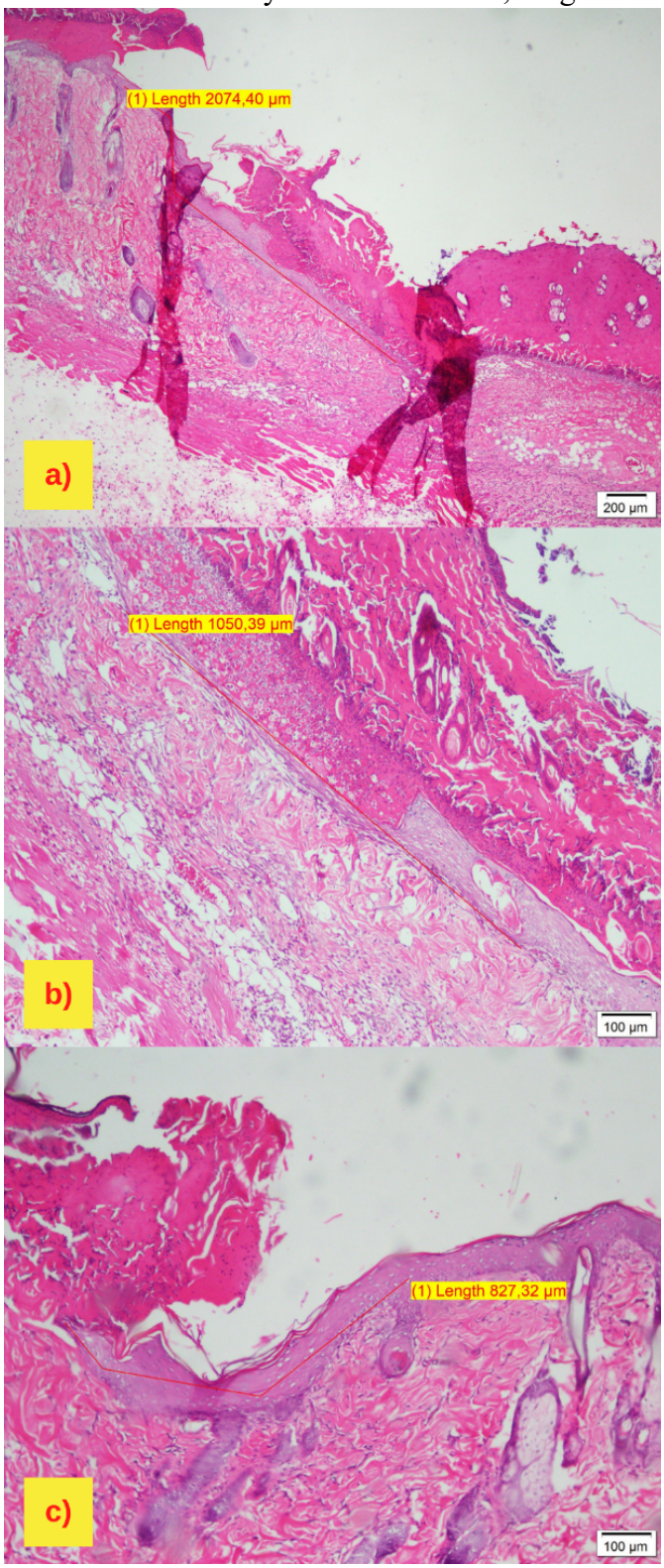

Figure 1. Histopathological description of re-epithelialization length with hematoxylin-eosin staining. a. hydrogel membrane with liquid smoke, b. topical liquid smoke, c. hydrogel membrane.

4. Discussions

Based on the results of the study and statistical analysis, significant results were obtained, which means that the data for each group gave different results on the length of re-epithelialization. This is because P1 has various properties that benefit the re-epithelialization process, namely antibacterial, antioxidant and anti-inflammatory.[14,16–18]

Wound exudate is a normal component of the wound healing process. Burns usually have excess exudate. One of the criteria for infected wounds is the increased amount of exudate.[19,20] Covering wounds with antibacterial agents can prevent and treat infections. Bacteria trapped in the exudate will be inhibited by antibacterial agents and absorbed by the wound dressing. Hydrogel membrane which has a high absorption capacity with liquid smoke as an antibacterial agent is a suitable combination to heal and prevent wounds from infection.[16,21,22]

Bacterial activity in the wound causes delays in wound healing due to the inflammatory response.23–25 In the control group, the results of the lowest re-epithelialization length were obtained. Although we did not perform an antibacterial test to determine whether the wound on control group was infected or not, it can be seen that control group had a slower wound healing process than other groups that had antibacterial agents, namely liquid smoke. This is in accordance with the results of previous studies which state that the hydrogel membrane without liquid smoke does not have antibacterial properties so that there may be bacterial activity in control group.[17]

TNF-α is a proinflammatory cytokine that is closely related to the wound healing process in the skin in the early phase. However, the prolonged presence of TNF-α will cause chronic wounds.[26–28] Research on TNF-α showed that the use of TNF-α inhibitors was able to inhibit wound closure and the inhibition only lasted until the third day.[26] Another study showed that giving TNF-α at an optimal dose every day promoted reepithelialization and angiogenesis, thereby accelerating wound healing compared to the control group and the TNF-α inhibitor group. Administration

of TNF-α that exceeds the optimal dose does not produce good angiogenesis and re-epithelialization.[28] Reducing the expression of NF-B which is one of the mechanisms to reduce TNF-α levels. Previous studies have shown that liquid smoke can reduce NF-κB expression more strongly than anti-inflammatory drugs.[14,29] Liquid smoke seems to be able to maintain optimal levels of TNF-α so that wound healing gets a good re-epithelialization process. This can be proven from the results of this study which showed that the administration of liquid smoke had a longer re-epithelialization than the group without liquid smoke. The group without liquid smoke may have TNF-α levels that exceed the optimal limit because they do not get anti-inflammatory agents.

Antioxidants play an important role in wound healing, namely as a regulator of ROS levels. Normal levels of ROS are required to resist invading microorganisms and convey cellular information. ROS stimulates the proliferation and migration of fibroblasts and keratinocytes thereby helping the re-epithelialization process. The function of antioxidants in wound healing cooperates with other properties such as antibacterial because chronic wounds are formed by pathological conditions that result in prolongation of inflammation and the creation of excessive ROS.[30–32] A study that tested antioxidant therapy in acute wounds said that the administration of antioxidants succeeded in healing acute wounds 8 times more than chronic wounds. Another study also proved that the administration of antioxidants in burns resulted in the best completeness of wound healing compared to other groups. Antioxidant administration also affects the expression of MMP1 which is required to support the re-epithelialization process.[32,33] This study has similarities with the results of this study which proves that the administration of liquid smoke which has antioxidant properties accelerates the process of re-epithelialization of burns.

Antibacterial, anti-inflammatory and antioxidant properties are needed in wound healing to balance the inflammatory process and prevent potential local infections so that the best wound healing results are obtained. These properties are owned by the liquid smoke combination hydrogel membrane. In this study, the best results were found for wound closure in the group given the hydrogel membrane combined with liquid smoke compared to the group given the liquid smoke and hydrogel membrane. This study certainly has limitations, namely burns of rats that are less than perfect due to the shape of the burns that are less uniform. In addition, the number of histopathological preparations did not describe the entire wound because each sample only counted one histopathological preparation.

5. Conclusion

Based on the results of the study, there was a significant difference in the length of re-epithelialization in burn rats given a hydrogel membrane with a combination of liquid smoke, hydrogel membrane and liquid smoke. The order of group average length of re-epithelialization from the longest to the lowest is the combined liquid smoke hydrogel membrane group (P1), the liquid smoke group (P2) and the hydrogel membrane group (K).

Ethical Approval

This research has been approved by Ethical Committee for Health Research, Faculty of Medicine, Diponegoro University with approval number 83/EC/H/FK-UNDIP/VIII/2021.

Conflicts of Interest

There is no conflict interest in this research

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Author Contributions

Conceptualization, A.R., V.K. and E.M; methodology, A.R., V.K. and E.M; software, A.R.; validation, A.R., V.K. and E.M; formal analysis, A.R.; investigation, A.R.; resources, A.R.; data curation, A.R.; writing—original draft preparation, A.R.; writing—review and editing, A.R., V.K. and E.M.; visualization, XXX; supervision, V.K. and E.M; project administration, A.R.; funding acquisition, A.R

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