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Histological Comparison of Lyophilized Amniotic Membrane, Tulle Gras Dressing and Topical Gentamicin on Acute Partial Thickness Wound, In Vivo Study

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Abstract

Background: Partial thickness wounds refer to the damage that extends from the epidermis to the upper layers of dermis. This study aims to compare the histological regrowth of acute partial thickness wounds following application of lyophilized amniotic membrane, tulle grass dressing and topical gentamicin. This study aims to establish a standard approach for treating partial thickness wound.
Methods: Three groups of Mus Musculus mice were used in our experimental study. Similar to harvesting split thickness skin grafts, partial thickness wounds are performed. The wound was then treated with lyophilized amniotic membrane, tulle grass dressing and topical gentamicin. Using the Scoring System for Histological Assessment of Wound Healing, the wound is histopathologically examined at day 14. In this study, One way Anova was used for statistical analysis.
Results: Application of lyophilized amniotic membrane showed different histological characteristic of healing from other method, with p=0.001 it showed significance epidermal growth compared to topical gentamicin. Whereas dermal thickness were also better, accompanied by lower dermal collagen density. Amniotic membrane has a large source of stem cells and contains several growth factors which is important in the physiological process of wound healing and tissue regeneration.
Conclusions: Characteristic of skin healing using lyophilized amniotic membrane showed rapid epidermal growth and gave impression of less fibrosis tissue, so that would be potential for better wound treatment especially for prevention of scar.

Keywords: lyophilized amniotic membrane, partial thickness wound, histopathological comparison, tulle dressing, topical gentamicin

Introduction

The skin was initially developed to interact with the external environment and perform various functions. It plays a crucial role in homeostasis, from maintaining thermal stability to sensing external stimuli. The skin primarily serves as a protective barrier, preventing dehydration and shielding the internal structures from mechanical, chemical, and thermal damage.[1] This defensive role extends to the complex barrier response of the immune system, which not only guards against pathogenic infections but also supports commensal microorganisms. Furthermore, the skin possesses effective mechanisms to seal any breaches in its barrier rapidly, a process known as the wound healing response.[1,2] The skin has evolved repair mechanisms that enable it to heal rapidly and effectively. Still, certain cellular aspects of each response to injury may be compromised, leading to impaired wound closure.[3]

Partial thickness wounds involve the loss of skin layers from the epidermis to the upper part of the dermis. These wounds include those resulting from skin graft removal surgery, excoriation injuries, and second-degree burns. According to a study conducted by Kepel et al. in 2019 at Prof. Dr. R. D. Kandou General Hospital in Manado, Indonesia, excoriation injuries were found to be the most common type of injury following motorcycle traffic accidents. Majorities of this type of wound can heal with proper wound care alone, without any surgical intervention, allowing the wound to close effectively.[4,5]

Current wound care practices to prevent infections use tulle (sterile gauze with antibiotics), gentamicin ointment, or amniotic membranes. Several studies have been conducted to compare the effectiveness of these methods for wound healing in terms of healing time and infection rate. Topical application of gentamicin always had much higher clinical efficacy levels than non-gentamicin applications. However, its use as prophylaxis for advanced infection in acute or chronic wounds is still debated in several countries.[6,7,8]

To this day, no studies have been conducted to compare the effectiveness of lyophilized amniotic membrane, tulle, or gentamicin ointment for the treatment of partial-thickness wounds in Indonesia, particularly at Dr. M. Djamil General Hospital. Therefore, there is no agreement in the form of standard operating procedures used at this hospital for partial thickness wound treatment. This study compares the histological regrowth of acute partial thickness wounds following the application of lyophilized amniotic membrane, tulle gras dressing, and topical gentamicin. Hopefully, in the long term, a standard approach for treating partial-thickness wounds might be established.

Methods

This is an experimental in vivo study with a post-test-only approach using a control group design. A total of 24 male rats (*Mus musculus*) were included. The rats ranged about 8-10 weeks old, weighing 20-30 grams, and were classified as healthy.

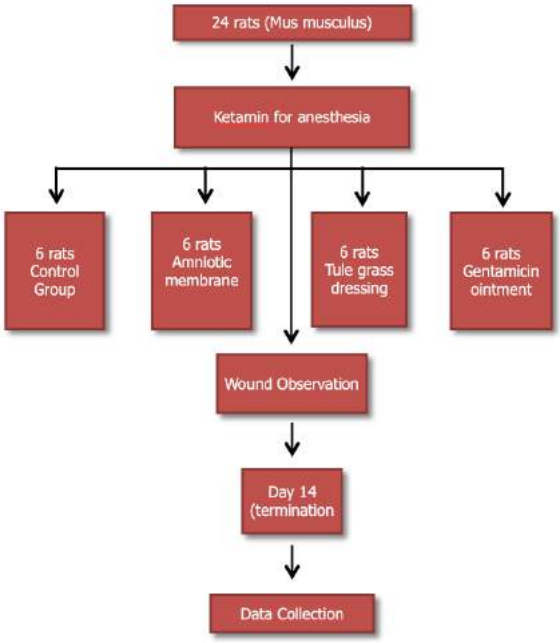

Figure 1. Experimental study flow diagram

The rats were randomized into four groups, each consisting of 6 rats (Fig. 1). The first 6 rats were grouped as controls, which were treated with 0.9% NaCl. The second group was treated using lyophilized amniotic membrane, the third group used tulle gas dressing, and the last group used 0.1% gentamicin ointment.

All of *Mus musculus* were given anesthesia with xylazine and ketamine intramuscularly. Then, the *Mus musculus* backs were shaved, followed by making partial thickness wounds performed similar to harvesting split-thickness

skin grafts. The wound size is 1 x 1 cm^2. The depth of the wound is characterized by bleeding spots appearing on the wound without any visible fat layer.

Table 1. Scoring System for Histological Assessment of Wound Healing

Characteristic	Score	Features
Edema	0	No evidence
	1	Focal presence at the wound margins
	2	Present in <50% of the wound tissue examined
	3	Present in >50% of the wound tissue examined
Leucocytes	0	No evidence
	1	Mild presence
	2	Moderate number of cells
	3	Prominent feature
Macrophages	0	No evidence
	1	Mild presence
	2	Moderate number of cells
	3	Prominent feature
Granulation tissue	0	No evidence
	1	Present at the wound margins
	2	Present in <50% of the wound tissue examined
	3	Present in >50% of the wound tissue examined
Fibroblasts	0	No evidence
	1	Present only in the perivascular spaces
	2	Present in <50% of the wound tissue examined
	3	Present in >50% of the wound tissue examined
Collagen	0	No evidence
	1	Focal presence in fibroblasts around new capillaries
	2	Moderate amount in the repair tissue
	3	Dominant feature
Epithelialization	0	No evidence
	1	Epidermal thickening and cell migration at wound margins
	2	>50% of wound epithelialized
	3	Epithelialization complete

Mus musculus was given 0.9% NaCl, lyophilized amniotic membrane (Fig. 2), tulle, and gentamicin ointment on the excised wounds, then later covered with gauze and adhesive tape. *Mus musculus* were monitored every day to make sure that the dressing stayed put. The wound dressing was replaced with the new ones every 3 days. The wound is histologically examined on the 14th day using the Scoring System for Histological Assessment of Wound Healing (Tab. 1).

Figure 2. Lyophilized amniotic membrane produced by Dr. M. Djamil General Hospital Tissue Bank Installation

Mus musculus then later was euthanized. After the rats died, the skin tissue was taken to the Anatomical Pathology Laboratory, Faculty of Medicine, University Andalas, and INA Lab to make histology preparations. The tissue samples were then fixed using 4% phosphate-buffered formalin for a minimum of 24 hours. Then, the tissue was cleaned with histoclear and embedded in paraffin. Wound tissue with a thickness of 4 mm was obtained using a microtome. The coloring was done using hematoxylin and eosin (H&E). The histological preparations were then observed under a light microscope using a CX 33 microscope. Photomicrographs were taken with a 3.1 MP Sony Exmor camera, CMOS, and the Betaview program.

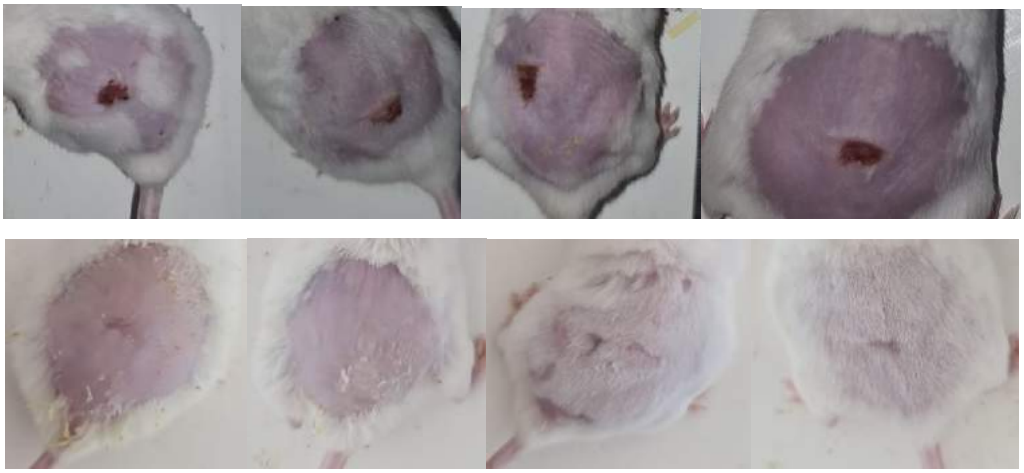

Figure 3. Mus Musculus partial thickness wounds on the 3rd day (above) and 14th day (below) following each treatment (in order from left to right, control group, lyophilized amniotic membrane, tulle, gentamicin ointment) where granulation tissue is no longer visible.

Skin tissue was measured quantitatively for the thickness of the epidermis and dermis, as well as semi-quantitative histologic parameters in the form of edema, leucocytes, granulation, fibroblasts, collagen, and epithelialization. The measurement of epidermal thickness was measured at 400x magnification by drawing a straight line from the basal epidermis to the upper border of the stratum granulosum under the corneum layer at 10 different points and displayed as an average value in µm. Dermis thickness was measured at 100x magnification by drawing a straight line from the basal epidermis to the lower level of the dermis at 10 different points and displayed as the average value in µm. Data analysis was carried out using SPSS version 25 software. Univariate analysis and bivariate analysis were carried out in this study. Univariate analysis was carried out to present the data distribution, meanwhile, bivariate analysis was carried out using One Way ANOVA to determine the relationship between variables, where $p<0.05$ is considered statistically significant.

Results

Twenty-four rats were divided into four groups, each group consisting of 6 mice. For histological parameters, assessment is carried out by measurement of epidermis and dermis thickness, as well as histological scoring including edema, leukocytes, macrophages, granulations, fibroblasts, collagen, and epithelium. The result exhibits that application of lyophilized amniotic membrane showed different histological characteristics of healing with $p=0.001$ after statistical analysis using One Way ANOVA, which means that there is significant epidermal growth compared to topical gentamicin. Dermal thickness showed no significant difference between each group, probably because this was a partial thickness wound affecting only part of the dermal layer so the measuring of dermal thickness could not be properly done. For edema, leucocyte, macrophage population, fibroblast, and granulation tissue forming, there was no significant difference between each group. Furthermore, the amniotic membrane group showed less collagen density than other groups.

Table 2. Histological results of partial thickness wound healing in Mus musculus based on the epidermis and dermis thickness, and histological score

Group	Mean thickness (µm)		Mean histological score						
	Epidermis	Dermis	Edema	Leukocytes	Macrophage	Granulation	Fibroblast	Collagen	Epithelial
Control	25.30	441.73	1.00	1.00	1.00	1.00	1.80	2.00	3.00
Gentamicin 0.1%	29.85	385.51	1.00	1.00	1.00	1.50	2.00	2.67	3.00
Tulle	15.38	408.42	1.50	1.00	1.00	1.00	1.00	1.00	3.00
Amnion	10.68	333.21	1.00	1.00	1.00	1.00	1.00	1.00	3.00

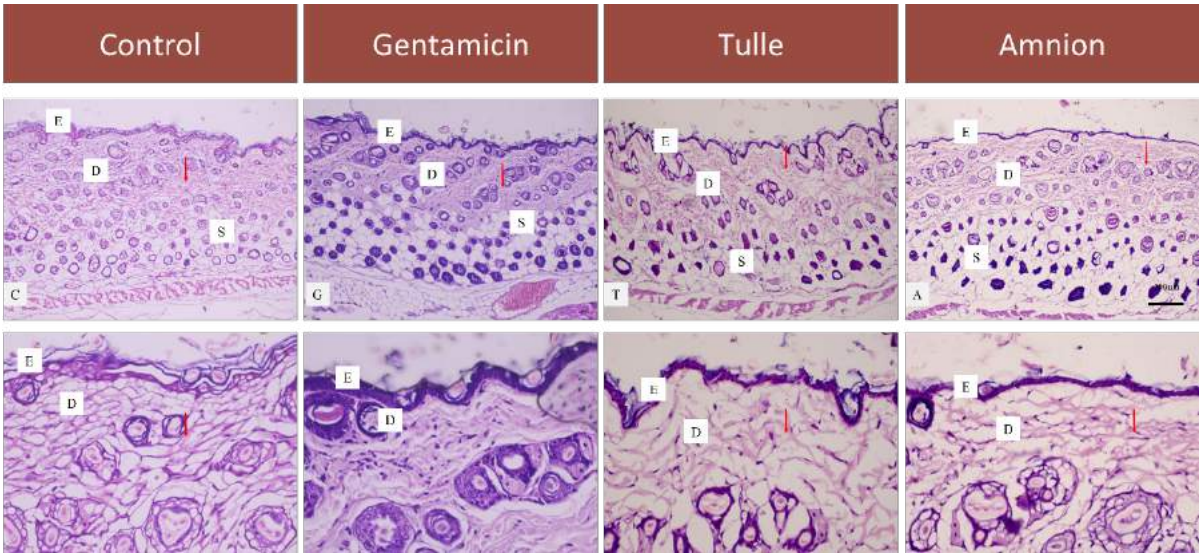

Figure 3. Histopathological results under a microscope after fourteen days. The upper picture shows the skin in its whole thickness, and the picture below shows only the epidermal and dermal layers as this is a partial-thickness wound. Top panel original magnification 100x, bottom 400x.

Discussions

Lyophilized amniotic membrane showed better histological outcomes compared to gentamicin ointment and tulle gras dressing. Human amniotic membrane has been used clinically in a variety of ways applications over the last 100 years and generates huge amounts of data in various fields of medicine. Its clinical use in wound closure for burns, chronic wounds, diabetic pressure injury, and conjunctival healing after pterygium repair has been proven to be beneficial and effective.[9,10] Several methods have been used for preserving the amniotic membrane such as fresh storage (stored at 4°C), cryopreservation (either at -86°C or using liquid nitrogen) and amnion radiation (Radiation Sterilized Lyophilized Amniotic Membrane) respectively aims to keep all network components as similar as possible with fresh tissue components and has a longer shelf life.[11] Amnion has unique properties due to its anti-inflammatory, bacteriostatic, and protective effects on wounds, as well as reducing scar tissue, reducing pain, and supporting epithelialization.[10,12]

This finding is similar to a study done by Shahid et al. in 2019 which reported that amniotic membrane was more effective as wound topical treatment in terms of healing time, level of infection, and less need for follow-up. [7] El Heneidy et al. in 2016 also reported that the amniotic membrane supports epithelialization corresponding to the suppression of excessive fibrosis.[13] Meanwhile, in a study conducted by Wang et al. in 2019 concluded that topical application of gentamicin ointment was associated with significantly higher rates of clinical efficacy and shorter duration of wound healing. However, its use as prophylaxis for advanced infection in acute or chronic wounds is still controversial in some countries.[8]

The amniotic membrane is an elastic and semi-permeable tissue made of 5 layers. The innermost layer is epithelial cells, which have a large source of stem cells.[14] Membrane human amniotic fluid also contains many *epidermal growth factors* (EGF), *basal fibroblast growth factor* (bFGF), keratinocyte growth factor, *transforming growth factor* (TGFa and TGFp), nerve growth factor hepatocyte growth factor, which is important in the physiological process of wound healing and tissue regeneration.[15,16,17] The amniotic membrane surface is non-adhesive which could prevent overgrowth and incidence of fibrosis. The hyaluronic acid found in the amnion membrane could provide a moist environment that can also decrease excessive fibrosis.[18,19,20]

This study was experimental research on animals, so it is difficult to assess another role of lyophilized amniotic membrane in wound healing along with its analgesic effects that can only be assessed in human subjects. Besides that, this study only assessed the histological level so the role of lyophilized amniotic membrane in the release of inflammatory cytokines in the wound healing process can not be analyzed.

Conclusions

Characteristics of skin wound healing using lyophilized amniotic membrane showed rapid epidermal growth and gave an impression of less fibrosis tissue, so amnion might have a better potential for wound treatment, especially for prevention of unfavorable scar formation. Further research on human subjects might be needed to assess the direct effects of amniotic membranes on human wounds including subjective variables like analgesic effect. Research needs to be carried out at the molecular level to identify the lyophilized amniotic membrane's role in scar formation and inflammatory cytokine migration.

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Declarations of competing interest

The authors declare no conflicts of interest.

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