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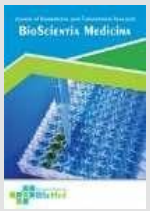

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Comparison of the Effectiveness of Lyophilized Amniotic Membranes in the Partial Thickness Wound Healing Process in *Mus musculus*

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A B S T R A C T

Background: Currently, tulle or gentamicin ointment is generally used to prevent wound infections in wound care. Human amniotic membrane has been used in medical fields such as wound care. So far, there is no comparative evidence of the effectiveness of each ingredient. This study aimed to compare the effectiveness of lyophilized amniotic membrane, tulle, and 0.1% gentamicin ointment in the wound healing process. partial thickness on *Mus musculus* clinically and histologically. **Methods:** This type of research is experimental with a post-test-only control group design. There were 24 rats divided into each group. This research was conducted at the anatomical pathology laboratory, Faculty of Medicine, Universitas Andalas, and INA lab in July - September 2023 using rats (*Mus musculus*), which were divided into 3 treatment groups and one control group. **Results:** Clinically, lyophilized amniotic membrane, 0.1% gentamicin ointment, and tulle provided better results than controls regarding erythema variables. Histologically, the results showed that the lyophilized amniotic membrane group had the thinnest epidermis and dermis, as well as better histological scores for granulation, fibroblasts, and collagen than the 0.1% gentamicin ointment, tulle, and control groups. In this study, significant differences in effectiveness were found in terms of erythema, skin thickness, granulation, fibroblast, and collagen histological scores, with better results in the lyophilized amniotic membrane group. **Conclusion:** Clinically, lyophilized amniotic membrane, tulle, and 0.1% gentamicin ointment were more effective than controls for wound treatment due to faster reduction in erythema. However, histologically, the lyophilized amniotic membrane was proven to be more effective than tulle, 0.1% gentamicin ointment, and control in terms of epidermis and dermis thickness, granulation level, fibroblasts, and collagen.

1. Introduction

Harsh external environments often result in injury to the skin, and it is therefore not surprising that our skin has advanced reparative processes that allow it to heal quickly and efficiently. Despite considerable innate reparative capabilities, some cellular aspects of an individual's injury response can be weakened, thereby compromising wound closure. This attenuation is most often the result of pathological systemic changes. Wound partial thickness is the loss of layers of skin from the epidermis to the upper part of the dermis. Excoriation wounds are the most common type of injury after motorbike traffic accidents. At this depth of the wound, it is hoped that the wound can heal only with wound care without surgery so that the wound can close.[1-5]

Human amniotic membranes have been used clinically in a variety of applications for more than the last 100 years and have generated a large amount of data in various fields of medicine. Its clinical use is

wound closure for burns, chronic wounds, diabetes wounds, and conjunctival healing after pterygium repair. There are several methods that have been used to preserve amniotic membranes, such as fresh storage (stored at 4°C), cryopreservation (either at minus 86°C or liquid nitrogen), and amnion radiation (Radiation Sterilized Lyophilized Amniotic Membrane), each of which aims to maintain all tissue components as similar as possible to fresh tissue components and have a longer shelf life. The grafting method using amnion for wound healing has unique properties due to its anti-inflammatory, bacteriostatic, wound protection, scar tissue reduction, and pain relief, as well as epithelialization effects.[6-10] This study aimed to compare the effectiveness of lyophilized amniotic membrane, tulle, and 0.1% gentamicin ointment in the wound healing process of partial thickness in *Mus musculus* clinically and histologically.

2. Methods

This study is an in vivo experimental research with a post-test-only approach with a control group design. A total of 24 rats (*Mus musculus*) male, 8-10 weeks old, weighing 20-30 grams, and healthy rats were included in this study. Then, randomization was carried out. The first 6 rats were grouped into the treatment group with wound treatment using lyophilized amniotic membrane, the second 6 rats with tulle, The third 6 rats were treated with 0.1% gentamicin ointment, and the other 6 rats were treated with 0.9% NaCl.

Rats *(Mus musculus*) were given anesthesia with xylazine and ketamine intramuscularly. Then, the rat's backs were shaved previously moistened with soapy water. Excision of the rats' backs using a number 10 scalpel like the extraction technique split-thickness skin grafts. The size of the wound is 1 x 1 cm in length and width. The depth of the wound down to the epidermis and part of the dermis is characterized by bleeding spots appearing on the wound and no visible fat layer. Rats *(Mus musculus*), which have been excised and then given lyophilized amniotic membrane, tulle, gentamicin ointment, and 0.9% NaCl, covered with gauze and then brown plaster. The rats were monitored by researchers every day so that the dressing did not come off. The wound dressing is opened every 3 days, and the amnion and tulle are replaced with new ones. Gentamicin ointment and 0.9% NaCl are applied to the wound. After being treated for 14 days, the rats were euthanized by means of decapitation, which was previously anesthetized, and after the rats died, the skin tissue was taken 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. After that, coloring is 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. Skin tissue was measured for the thickness of the epidermis and dermis quantitatively, as well as semi-quantitative histologic parameters in the form of edema, leucocytes, granulation, fibroblasts, collagen, and epithelialization using the "scoring system for histological assessment of wound healing". The measurement of epidermal thickness of the epidermis 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 for each test variable. Bivariate analysis was carried out to determine the relationship between test variables, where $p<0.05$.

3. Results

In Table 1, there are significant differences between the control group, gentamicin ointment, tulle, and lyophilized amniotic membrane in histological parameters, namely epidermis thickness, granulation, fibroblasts, and collagen. In terms of skin thickness parameters, it was found that amnions had the thinnest epidermis and dermis compared to other groups. In the histological score parameters, there were three variables that had significant differences, namely granulation, fibroblasts, and collagen, where the mean score was found to be the highest in the gentamicin ointment group, followed by the control group.

Table 1. Comparison of the effectiveness of lyophilized amniotic membrane, tulle, and 0.1% gentamicin ointment on partial thickness wound healing in rats (*Mus musculus*) based on histological parameters.

Clinical criteria	Group				p-value
	Control	Gentamicin	Tulle	Amnion	
Thickness					
Epidermis	25,30±9,31	29,84±4,01	15,37±3,90	10,41±1,19	0,000[a]
Dermis	427,84±105,85	385,50±40,33	408,42±12,60	333,21±33,97	0,023[b]
Histological score					
Edema	1,00±0,00	1,00±0,00	1,50±0,54	1,33±0,51	0,088[b]
Leukocytes	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00	-
Macrophages	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00	-
Granulation	1,00±0,00	1,50±0,54	1,00±0,00	1,00±0,00	0,020[b]
Fibroblast	1,67±0,51	2,00±0,00	1,00±0,00	1,00±0,00	0,000[b]
Collagen	2,00±0,00	2,67±0,51	1,00±0,00	1,00±0,00	0,000[b]
Epithelium	3,00±0,00	3,00±0,00	3,00±0,00	3,00±0,00	-

[a]one-way ANOVA
[b]Kruskal Wallis

4. Discussion

The effectiveness of lyophilized amniotic membrane, tulle, and 0.1% gentamicin ointment was clinically better than the control, as seen from the degree of erythema. Meanwhile, histologically, the effectiveness was found to be higher only for lyophilized amnion membrane and tulle, as seen from the thickness of the epidermis, dermis, and histological scoring (granulation, fibroblasts, and collagen). Overall, the lyophilized amniotic membrane had the highest effectiveness compared to tulle, gentamicin ointment, and control, as seen from clinical erythema that faded more quickly, the thickness of the epidermis and dermis was the thinnest, and the smallest histological score of wound healing related to granulation, fibroblasts, and collagen.

The amniotic membrane is an elastic and semi-permeable tissue made of 5 layers. The innermost layer, closest to the fetus and in direct contact with amniotic fluid, are epithelial cells.[11-13] These cells have certain characteristics that make them a sizable source of stem cells. Human amniotic membranes contain many growth factors such as epidermal growth factor (EGF), basal fibroblast growth factor (bFGF), keratinocyte growth factor, transforming growth factor (TGFa than TGFp), nerve growth factor, and hepatocyte growth factor, which is important in the physiological processes of normal wound healing and tissue regeneration. In this study, the thinnest epidermis thickness was found in the amnion group.[14-16] This is due to the properties of the amniotic membrane, which reduces the formation of scar tissue. One surface of the amnion membrane is non-adhesive, preventing overgrowth and reducing the incidence of fibrosis. The hyaluronic acid found in the amnion membrane also prevents excessive fibrosis.[17-20] Viewed histologically, the scoring for granulation, collagen, and fibroblasts is also the smallest, namely 1.00 ± 0.00 for the amniotic membrane, where these

three parameters also determine the thickness of the epidermis.

5. Conclusion

The administration of lyophilized amniotic membrane, tulle, and 0.1% gentamicin ointment was proven to be more effective than the control in wound healing. the partial thickness of *Mus musculus* clinically in terms of erythema and histologically in terms of epidermis and dermis thickness, and granulation, fibroblast, and collagen histological scores.

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