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RELATIONSHIP BETWEEN DRIED AMNION SHEET AND DRY POWDERED AMNION WITH CHANGES IN WOUND AREA IN ACUTE WOUNDS

Ferdinand[1,2]*, Afriwardi[3], David Sontani Perdanakusuma[4], Hirowati Ali[5]
[1]Doctoral Student of Biomedical Sciences, Faculty of Medicine, Universitas Andalas, Padang, Jl. Perintis Kemerdekaan No.94, Padang Timur, Padang 25129, Indonesia
[2]EstetiQ Clinic Pekanbaru, Jl. Melati No.28, Padang Bulan, Senapelan, Pekanbaru, Riau 28155, Indonesia
[3]Department of Physiology, Faculty of Medicine, Universitas Andalas, Jl. Perintis Kemerdekaan No.94, Padang Timur, Padang 25129, Indonesia
[4]Department of Plastic, Reconstructive and Aesthetic Surgery, Dr. Soetomo General Hospital, Faculty of Medicine, Universitas Airlangga, Jln Dr. Ir. H. Soekarno, Mulyorejo, Surabaya, East Java 60115, Indonesia
[5]Department of Biochemistry, Faculty of Medicine, Universitas Andalas, Jl. Perintis Kemerdekaan No.94, Padang Timur, Padang 25129, Indonesia
*ferdinandneurologic@yahoo.com

ABSTRACT
Dried sheet amnion has long been used as a wound treatment that provides better results than standard general care. In addition to dried amnion sheet, dry powdered amnion preparations have begun to be used for wound care. This study aims to evaluate the effectiveness of dry powdered amnion compared to dried amnion sheets in wound healing, and to compare both treatments with standard acute wound care. Sixty-three healthy Wistar rats were randomly divided into three groups: dried amnion sheet treatment group, dry powdered amnion treatment group, and standard wet wound care control group. The research procedure began with the administration of anesthesia using penthotal at a dose of 10mg/kgBW intramuscularly. The backs of the rats were shaved and a 2x1cm wound was designed using a GOAL razor blade after disinfection with 10% betadine solution and 1:30 savlon. Wounds were made on the backs of the rats, and treatments were given according to the group. The wound area was evaluated at time points 6 hours after treatment, day 2 and day 5. Statistical analysis was performed to compare the effectiveness of the treatments. Both treatments with dry powdered amnion and dried amnion sheets significantly reduced the wound area compared to the control group. However, treatment with dried amnion sheets showed higher efficacy in reducing wound area compared to treatment with dry powdered amnion. Statistical analysis confirmed significant differences in wound area reduction between groups. Dry powdered amnion and dried amnion sheets were effective in reducing wound area compared to standard wet wound care. Treatment with dried amnion sheets showed greater efficacy than treatment with dry powdered amnion.

Keywords: amnion; dry powdered amnion; dried amnion sheet; wound healing

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INTRODUCTION

Wounds remain a significant global problem, affecting both direct and indirect morbidity and economic burden (Sen, 2021). It is estimated that around 1-2% of the total population worldwide suffers from chronic wounds, highlighting the urgency of wound healing management (Nussbaum et al., 2018). The wound healing process often results in scarring, which can cause a variety of physical, social, psychological, and aesthetic problems for sufferers (Ogawa, 2017). Superficial wounds tend not to produce significant scars compared to deep wounds, but scars such as keloids and hypertrophic scars can result in complications such as contractures, pruritus, and pain, significantly affecting the quality of life of sufferers (Chiang et al., 2016). In general, disorders such as keloids and hypertrophic scars are the result of impaired and incomplete wound healing processes. Therefore, further research in the field of wound healing and scar management is essential in addressing this global health problem (Lee & Jang, 2018).

Although the wound healing process involves a complex interaction of many factors, it can generally be divided into four overlapping and clearly distinguishable phases (Morton & Phillips,

2016; Reinke & Sorg, 2012). The first phase is the homeostasis phase, which occurs within minutes to hours after injury. This is the period in which the body attempts to stop bleeding and regulate blood clotting factors. The second phase is the inflammatory phase, which occurs around days 1-3 after injury. In this phase, an inflammatory response occurs with the release of inflammatory mediators to clean the wound area from pathogens and dead cells. The third phase is the proliferation phase, which occurs around days 4-21 after injury. In this phase, new cells proliferate and granulation tissue is formed to repair the wound. The last phase is the remodeling phase, which occurs around days 21-365 after injury. In this phase, scar tissue begins to undergo remodeling and structural changes to obtain optimal strength and elasticity. Disruption in any of these phases can result in delayed wound healing or excessive scar tissue formation (Landén et al., 2016).

In addition to sheet form, amnion preparations are also available in powder form. The majority of the use of dry powdered amnion (DPA) is for injection therapy aimed at reducing the local inflammatory response (Salazar-Noratto et al., 2019). There are two case reports of the use of amnion powder on wounds (Hawkins, 2016; Murphy et al., 2020b). Hawkins et al. (2016) used ABK to treat diabetic wounds and noted better wound healing results and minimal reduction in contraction. The use of dry powdered amnion has been shown to be effective in healing chronic wounds that are unresponsive to standard wound care therapy. In addition, wounds treated with amnion powder produced a satisfactory healing process and provided a good scar appearance.

Based on the advantages of dried amnion sheet preparations in powder form, researchers aim to assess their effectiveness in wound healing compared to a control group that received standard moist wound care. Thus, this study will compare the wound healing outcomes between patients who used powdered amnion preparations with those who received standard moist wound care. This step is important to evaluate the potential of dry powdered amnion as a more effective alternative in improving the wound healing process and producing better results compared to existing standard care. This study aims to evaluate the effectiveness of dry powdered amnion compared to dried amnion sheets in wound healing, and to compare both treatments with standard acute wound care.

METHOD

This study has gone through a review process and obtained approval from the Ethics Committee of the Faculty of Medicine, Andalas University, West Sumatra with the ethics letter number 1094/UN.16.2/KEP-FK/2022. Dried amnion sheet preparations were obtained from the Surabaya Tissue Bank and processed into powder according to the powdering standards set by the Faculty of Pharmacy, Andalas University, West Sumatra. Lomatulle brand vaseline gauze was used as a control material. A total of 63 healthy 3-month-old Rattus norvegicus wistar rats that had been acclimatized for 2 weeks were used in this study. The rats were then randomized and grouped into 3 treatment groups. The first group received treatment with dried amnion sheets, the second group received treatment with dry powdered amnion, and the third group received control treatment with tulle.

The research procedure began with the administration of anesthesia using penthotal at a dose of 10mg/kgBW intramuscularly. The backs of the rats were shaved and a 2x1cm wound was designed using a GOAL razor blade after disinfection with 10% betadine solution and 1:30 savlon. The first treatment with dry powdered amnion involved covering the wound with dry sheet amnion covering the entire wound surface. The second treatment with dry powdered amnion involved sprinkling dry powdered amnion to cover the wound surface. The third treatment involved covering the wound using tulle. The entire wound was then covered with thick sterile gauze and fixed with sutures on the back of the rat. All rats were given intramuscular injection of Penicillin Procaine at a dose of 100mg/kgBW. The rats were then kept in their respective cages with the same food in the type and amount that had been determined.

Figure 1. Before and after control group. Day 5

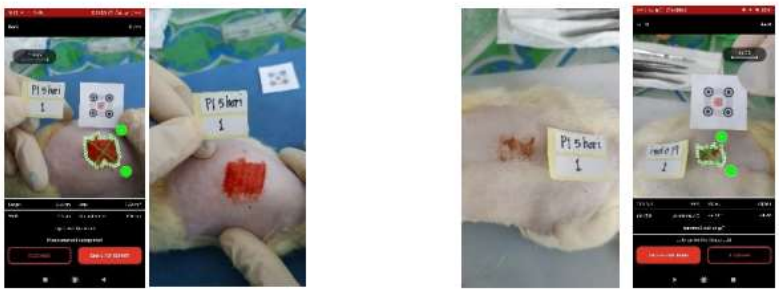

Figure 2. Before and after dried amnion sheets group. Day 5

Figure 3. Before and after dry powdered amnion group. Day 5

Wounds were evaluated at 6 hours after treatment, 2 days after treatment, and 5 days after treatment. Wound area was calculated before and after treatment. To conduct the evaluation, Rat were sacrificed using the decapitation technique. Wound area was measured visually using the IMITO® Android application. Data on wound area before and after treatment were recorded for statistical analysis using SPSS 23.0. Tested for normality through Shapiro-Wilk, then analyzed using two-way ANOVA with post hoc test for normal data or Kruskal-Walls for abnormal data, and presented in tables and graphs to support interpretation of the results.

RESULT

After collecting the results of the wound area calculation, the data were grouped in a table for statistical analysis. It is known that the number of treatments for each group is 21, therefore, the decision was made to conduct a data normality test using the Shapiro-Wilk technique to determine whether the data can be considered to come from a normal distribution, which is a prerequisite for conducting parametric statistical tests.

Table 1.
Normality Test using Shapiro-Wilk

		Shapiro-Wilk		
	Groups	Statistic	df	Sig.
WOUND AREA	Control (before)	.952	21	.366
	Control (after)	.981	21	.944
	DSA 1 before	.974	21	.815
	DSA 2 after	.960	21	.512
	DPA 1 before	.952	21	.372
	DPA 2 after	.914	21	.066

Based on the table results, the significance value for each group shows a normal distribution, with a value greater than 0.05. Thus, the prerequisites for the normality test are met. Therefore, the test of wound area before and after the action can be continued using the paired T-test. This step will allow for a statistical comparison between the wound area before and after treatment in each group, taking into account that the data tested comes from a normal distribution.

Table 2.
Paired T-test

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Control before	3.902381	21	.7697006	.1679624
	Control after	2.092857	21	.8230258	.1795989
Pair 2	DSA 1 before	3.444762	21	.7056955	.1539954
	DSA 2 after	1.930000	21	.9113726	.1988778
Pair 3	DPA 1 before	3.497619	21	.8760931	.1911792
	DPA 2 after	1.861429	21	.8188058	.1786781

In all treatments, there was a change in wound area before and after treatment. In the control group, there was a decrease in the average wound area from 3.9 cm^2 to 2.09 cm^2. While in the treatment with Dried Amnion Sheets, there was a decrease in the average wound area from 3.44 cm^2 to 1.93 cm^2. In the treatment with Dried Amnion Powder, there was a decrease in the wound area from 3.49 cm^2 to 1.86 cm^2. These changes indicate the potential effectiveness of the use of dried amnion sheets and dried amnion powder in reducing wound area and supporting the wound healing process.

Table 3.
Paired Sample T-Test

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Control before – Control after	1.8095238	1.2238075	.2670567	1.2524533	2.3665943	6.776	20	.000
Pair 2	DSA 1 before – DSA after 1	1.5147619	.8603524	.1877443	1.1231342	1.9063896	8.068	20	.000
Pair 3	BSA 1 before – BSA 2 after	1.6361905	.9828605	.2144777	1.1887977	2.0835832	7.629	20	.000

From the results of the paired sample T-test, the average difference in wound area in the control group was 1.8, the difference in the dried amnion sheet group was 1.51, and the difference in the dry powdered amnion group was 1.63. The significance value for all groups was less than 0.05, indicating that the difference in wound area in each group was significantly different.

The results of the T test showed that the calculated T value of each group was greater than the relevant T table value. The T table value used was 2.086. The calculated value for the control group was 6.776, for the dried amnion sheet group was 8.068, and for the dry powdered amnion group was 7.629.

Thus, the conclusion is that the treatment in both the control group, dried amnion sheet, and dry powdered significantly changed the wound area. Because the results were equally significant, the effectiveness in reducing the wound area was measured based on the highest T value. Therefore, of the three treatments, dry sheet amnion has the highest T value (8.068), while the control group has the lowest T value (6.776). This shows that dried amnion sheets has the highest effectiveness in reducing wound area compared to the control group and dry powdered amnion group.

DISCUSSION

Amnion has been known as a wound dressing since Davis first used it in 1910. Since then, research and application of amnion have spread throughout the world and have proven effective as biological wound dressings (Colocho, 1974; Robson & Krizek, 1973). The amniotic membrane is the innermost part of the placenta that is directly attached to the fetus. This membrane has a physical protective function and also contains various bioactive ingredients, so it is often used as a preparation in wound care (Dadkhah Tehrani et al., 2021). The use of amniotic membrane has long been used to treat various types of wounds, both chronic and acute (McQuilling et al., 2019). The advantages of amniotic membrane in wound care include the active biological content that helps the wound healing process and its physical protective function (McQuilling et al., 2019).

The anti-fibrotic, pro-angiogenesis, and pro-epithelialization properties have made amnion a material for wound healing, corneal healing, and tissue engineering. Its active ingredients include cytokines, extracellular matrix (ECM) materials, and other additional proteins (see Figure 2.3). Amnion preparations are released from their epithelium to prevent immunological rejection. In addition, efforts to clean the amnion sheet from living cells are carried out to reduce the potential for rejection with various substances such as Sodium Duodecyl Sulfate (SDS), Urea, EDTA, and trypsin (Ilic et al., 2016). Amnion can be stored fresh, frozen, and dried; and research has proven effective for wound care. One of the most popular forms is freeze-dried and preservation with glycerol (Lacorzana, 2020). This shows the flexibility of amnion as a therapeutic material that can be adapted according to medical care needs.

The application of dried amnion membrane has been shown to be beneficial for treating diabetic ulcers by reducing the wound area by 60% in the first use of amnion membrane (Boyar & Galiczewski, 2018). The physical properties of the amnion, such as elasticity, stiffness, and tear resistance, are closely related to the composition of the placenta. The orientation of collagen fibrils in the extracellular matrix is responsible for its strength and tear resistance. Its elastic ability is related to elastin and laminin fibers (Mamede & Botelho, 2015), as well as hyaluronic acid and glycosaminoglycans (Friel et al., 2017). Several studies have shown that the tear resistance strength of the amniotic membrane ranges from 100 to 400 Pa (Chen et al., 2012). Amnion membrane that has been cleaned of cells (decellularization) shows higher resistance, which is caused by the drying and shrinkage process (Chen et al., 2012).

The main problem in wound healing is the disruption of the transition from the inflammatory phase to the proliferative phase, which is often accompanied by changes in macrophage polarization from type M1 to M2 (Kloc et al., 2019). An imperfect transition to the proliferative phase can disrupt the wound epithelialization process, as well as cause the formation of myofibroblasts that are responsible for excessive wound contraction and unsatisfactory scar formation (Kloc et al., 2019). Interventions aimed at regulating the inflammatory response in wounds are expected to improve the wound healing process and produce better scar tissue (A. L. Moore et al., 2018). The main growth factor that plays an important role in the wound epithelialization process is Epidermal Growth Factor (EGF), which functions to stimulate the proliferation and migration of epithelial cells to close the wound (Nanney, 1990). The importance of EGF in this process is also related to its production by M2 type macrophages (Delavary et al., 2011). The external use of EGF preparations has been shown to provide optimal results in healing acute wounds, as well as other growth factor preparations such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and transforming growth factor (TGF) (Park et al., 2018). Interestingly, all of these growth factors are also present in amnion preparations as part of their biological content (M. C. Moore et al., 2020). Thus, the use of amnion in wound care can provide significant benefits by providing the growth factors needed to support the healing process.

Freeze-dried preserved amnion membrane, or dehydrated amnion membrane, is known to contain various cytokines and chemokines that play an important role in the wound healing process (Koob et al., 2014; Roy & Griffiths, 2020; Shah, 2014; Zelen, 2013). Dehydrated amniotic membrane contains a number of growth factors such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), placental growth factor (PLGF), granulocyte colony-stimulating factor (GCSF), anti-inflammatory interleukins (4, 6, 8, 10), and Tissue Inhibitor Metalloproteinase (TIMP) (Arpino et al., 2015; Koob et al., 2013; Lei et al., 2017a; Visse & Nagase, 2003; Werner & Grose, 2003). Amniotic membrane is also known to not cause an immune response (non-immunogenic), has anti-inflammatory properties, and has antibacterial activity (Ueta et al., 2002; Hao et al., 2000; Shimmura et al., 2001). In addition, amnion membrane provides a matrix that supports cell migration and proliferation by containing more than 220 growth factors, cytokines, and chemokines that support the wound healing process (Koob et al., 2014; Tenenhaus, 2017). Dried amnion in the form of a membrane (Amniotic Membrane Dry) is sometimes less flexible to adhere optimally to the wound surface, resulting in suboptimal release and contact of bioactive substances (Zheng et al., 2017). Therefore, thinking has led to the use of amnion preparations in powder form (Amniotic Powder), which is easier to use especially on irregularly shaped wounds (Hawkins, 2016). Dry powdered amnion (Amniotic Powder Dry) is known to have the advantage of releasing more bioactive substances compared to Amniotic Membrane Dry (Russo et al., 2012). These bioactive substances have a significant effect on the wound healing process and help in better scar formation (Lei et al., 2017; Murphy et al., 2020).

The advantage of the powder form is its ability to release more bioactive substances compared to the sheet form (Russo et al., 2012b). However, statistically, clinical effectiveness shows that dry sheet amnion provides better results. The advantage of the sheet form is its ability to provide better protection against wounds compared to the powder form, because it is able to provide denser protection that cannot be provided optimally by the powder form (Bose, 1979). This may explain why dried amnion sheet has the advantage of maintaining the wound bed optimally for the wound healing process. The results of the study showed that the process of reducing the wound area was better in the sheet form compared to the powder form.

CONCLUSION

Dry powdered amnion and dried amnion sheets were shown to be effective compared to controls in reducing wound area. Statistically, wound area reduction was better in the dried amnion sheet treatment. However, further research is needed to explore why healing with dried amnion sheets is more clinically effective than the powder form (Ahmed, 2023). In addition, further research into cellular biological responses is highly recommended to explain the wound healing process using dried amnion sheet and dry powdered amnio preparations. Thus, a deeper understanding of the mechanism of action and clinical differences between the two forms of preparation can be obtained to improve the effectiveness of wound care.

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