Sys Rev Pharm 2020;11(5):583-586
A multifaceted review journal in the field of pharmacy

Differences of Dried Amniotic Membrane Effect on Healing of Gastric Tissue Post Penetrating Trauma Reviewed from Collagen Thickness and Fibroblast Count

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ABSTRACT

Dried amniotic membrane (AM) has been shown to have the potential to help and facilitate proper closure of gastric tissue defect post penetrating injury trauma. This study evaluated the difference of fibroblast count and collagen thickness in gastric tissue post penetrating injury using with and without dried AM as a biological dressing. A randomized experimental study on New Zealand rabbits treated to an artificial gastric penetrating injury. The samples were divided into two groups, control and treatment, without and with dried AM, respectively. Microscopic evaluation of the gastric tissue specimen was done using hematoxylin-eosin staining. Average fibroblast count in the control group was 70.90±10.26, and the group with dried AM was 91.00±10.82 (p = 0.000). The mean thickness of collagen in the control group was 3142.48±1271.29 µm, and the group with dried AM was 4703.71±1807.88 µm (p = 0.006). These results showed significant difference in fibroblast count and collagen density when gastric penetrating injury was treated with primary closure and dried AM. There is a significant difference in the number of fibroblasts and collagen thickness in the repair of gastric rupture using dried AM as biologic dressing compared to the control group.

Keywords: dried amniotic membrane, fibroblast, collagen

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INTRODUCTION

Sharp trauma to the abdomen is one of the causes of visits of adult trauma patients to the emergency department (1). The procedure involving the abdominal cavity is an open surgery (2). Therapy for sharp trauma to the abdomen, especially the gastric part, continues to develop along with technological development (3). Nevertheless, the risk of leakage after closure can still occur. Various studies have been conducted to reduce the risk of leakage, including the use of various surgical techniques and materials. One of the special concerns is the use of local materials that can accelerate healing or reduce the risk of leakage (4).

The whole process of wound healing involves a series of complex events which can take a long time (5). In normal condition, wound can heal by itself, but how fast it is can be affected by many factors (6). Various methods to improve wound healing have been applied, one of which involves placing the amniotic membrane in the wound (7). Amniotic membrane is a material widely used to help to stimulate the healing process. In its development, the amniotic membrane was found to have many uses in the treatment of burns, reconstruction of the oral cavity, bladder, vagina, tympanoplasty, arthroplasty, and many more (8). The amniotic membrane also has other advantages because of its ability to reduce scar tissue formation and inflammation, accelerate healing time, and function as a scaffold for cell proliferation and differentiation [1]. The extracellular matrix component of the amniotic membrane also has a growth factor, so the amniotic membrane is considered a good candidate as a natural scaffold in tissue engineering. The amniotic membrane itself is a biomaterial that is easily obtained, processed, and easy to carry (9).

The amnion membrane is the innermost layer of 3 layers that make up the placenta by three layers: one epithelial layer, a thick basement membrane, and a vascular mesenchymal layer (10). Amniotic membranes contain basement membrane components, growth factors, and proteinase inhibitors (11). Previous studies have shown that amniotic membrane is a prosthetic material with anti-bacterial properties, low immunogenicity, can help epithelialization and wound healing, inhibit inflammation and scar formation, and increase angiogenesis (10). Amniotic membranes are also easy to obtain, easily processed and distributed. Amniotic membranes are obtained during labor by elective cesarean section and do not need to kill human embryos for isolation [2], so controversies such as the use of human cells can be avoided (11). Amniotic membrane is widely used to stimulate the wound healing process but has never been used to repair a ruptured gastric. This study aimed to examine the effects of amniotic membrane on gastric rupture repair in rabbits experimentally [3].

METHODS

This was an experimental study with a randomized control trial study design using 42 rabbits. The research samples were New Zealand white rabbits. The inclusion criteria of this study were rabbits aged between 6 to 9 months, body weight between 1900 to 2500 grams, and no visible anatomical abnormalities. Subsequent samples were divided into two groups, and those were the primary repair group of gastric rupture without using a dried amniotic membrane (AM) as the control group and the group using dried AM. In both groups, a 2 cm wide rupture was done with a width of 0.5 cm with the entire depth of the gastric wall and covered using 5/0 polypropylene monofilament yarn with one-on-one stitching. In the amnion membrane group, the repair was given a human amnion with a width of 4cm x 2cm fixed with the gastric mucosa. All experimental animals were treated in accordance with Universitas Airlangga Animal Care and Use Committee rules and had received research ethics no. 2.KE.032.02.2019.

From both groups, specimens were sacrificed on day 7. The 5 cm rupture repair segment was cut and fixed in 10% formaldehyde by histopathological examination to see the number of fibroblasts and collagen thickness in both groups of experimental animals. The data obtained were nominal in the study. Then, it was recorded, analyzed, and compared with the control and treatment groups with the Fishers Exact Test nonparametric test. The statistical data processing program used is the SPSS 16 program.

RESULTS

Table 1 shows that the mean age of the study sample in the control group is 7.52 ± 0.93 months, and the group using dried AM was 7.76±0.83 months (p = 0.416). The weight in the control group was 2171.43±170.71 grams, and the group using MAK was 2252.38±183.36 grams (p = 0.557). Characteristics of the sample in the basic data of the study showed that there were no significant differences in characteristics between the variables rabbit's weight and age in the two sample groups.

Table 1: Characteristics of Research Subjects

Characteristics	Groups		p
	Control	Treatment	
Age (month)			0.416
Mean±SD	7.52±0.93	7.76±0.83	
Median (min-max)	8 (6-9)	8 (6-9)	
Weight (gram)			0.557
Mean±SD	2171.43±170.71	2252.38±183.36	
Median (min-max)	2200 (1900-2500)	2300 (1900-2500)	
Number of Fibroblast			0.000
Mean±SD	70.90±10.26	91±10.82	
Median (min-max)	70 (54-92)	92 (70-117)	
Thickness of collagen			0.006
Mean±SD	3142.48±1271.29	4703.71±1807.88	
Median	2952	4480	

Table 1 shows that the mean number of fibroblasts in the control group was 70.90±10.26, and the group using dried AM was 91.00±10.82. Meanwhile, the mean thickness of collagen in the control group was 3142.48±1271.29 µm, and the group using dried AM was 4703.71±1807.88 µm. The comparative distribution of research results of the two variables is shown in Figures 1 and 2. Statistical test results show a significant difference in the number of fibroblasts and collagen thickness in the two groups (p = 0.000 and p = 0.006, respectively).

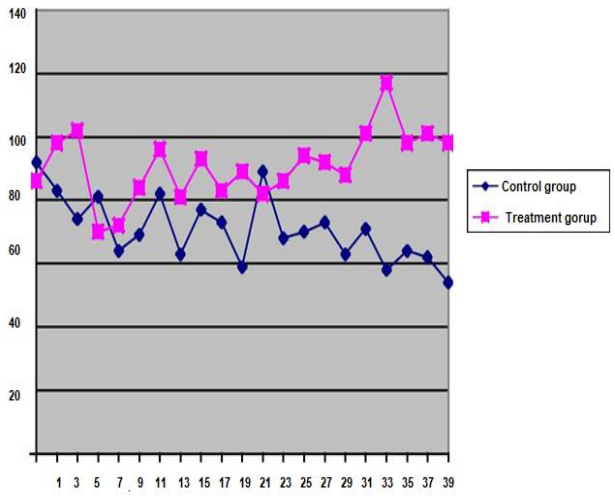

Figure 1. Comparative Graph of the Number of Fibroblasts in Research Samples

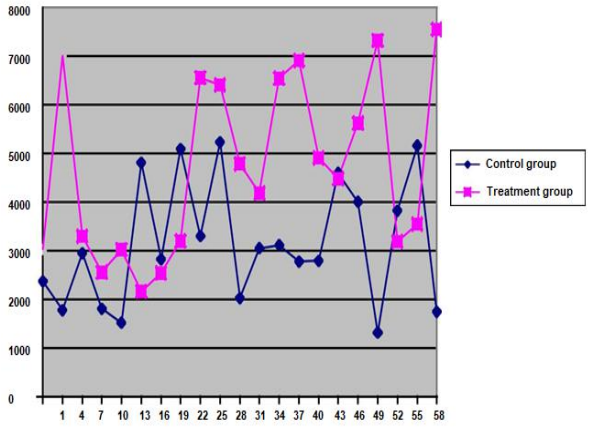

Figure 2. Comparison Graph of Collagen Thickness in Research Samples

DISCUSSION

The present study shows significant differences in the use of dried AM as biologic dressing compared with primary repair of gastric rupture (control group) in experimental rabbits. Gastric repair describes several systemic, local, and operative factors affecting the wound healing process. Wound healing is a form of cellular response to disruption or injury to tissue and involves activation of keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets. This process includes regular cell migration and the involvement of endothelial cells for angiogenesis. There are various growth factors (growth factors) and cytokines that are released to maintain wound healing. After homeostasis is achieved, the wound will undergo four healing phases: the inflammatory phase, the epithelialization phase, the proliferation phase, and the maturation phase, where in the proliferation phase, fibroblasts have a significant role for tissue proliferation and collagen synthesis (12,13).

Amniotic membrane has advantages because it has the ability to reduce the formation of scar tissue and inflammation, accelerate healing time, and function as a scaffold for cell proliferation and differentiation (14). The extracellular matrix component of the amniotic membrane also has a growth factor. Thus, the amniotic membrane is considered a good candidate as a natural scaffold in tissue engineering [4]. The amniotic membrane also reduces exudate by sticking tightly to wounds and acts as an anti-inflammatory due to a large amount of material blocking inflammatory mediators, such as proteases (9). The anti-inflammatory effect of the amniotic membrane suppresses pro-inflammatory cytokines

IL-1α and IL-β and produces natural metalloprotease (MMP's) inhibitors (15). Effects that actively suppress the proliferation of T lymphocytes and inhibit monocyte differentiation. Amniotic membrane has a natural inhibitor on the matrix able to stabilize the expression of matrix metalloproteinase in the inflammatory environment with a vital role in the healing process (16,17).

The previous study have shown a significant association with the use of dried AM as a wound healing, where dried AM can provide a matrix for cellular migration and proliferation, promote healing through various processes, and contain natural biological barrier to external contaminants. (18). Other studies show that amnion membranes not only function for wound healing but also tissue repair and regenerative therapy containing growth factors, such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), vascular EGF (VEGF), transforming growth factors (TGFs), nerve growth factor (NGF), and many chemokines known to be essential for wound healing (19-25). These growth factors are present in granules in concentrated platelet-rich plasma (PRP) (20,21). So through this study and in accordance with other studies, we have found that there is a significant relationship between the use of dried AM as biologic dressing compared with primary repair of gastric rupture without using dried AM.

CONCLUSION

There is a significant difference in the number of fibroblasts and collagen thickness in the repair of gastric rupture using dried AM as biologic dressing compared to the primary repair of gastric rupture without using dried AM.

REFERENCES

1. Butt MU, Zacharias N, Velmahos GC. Penetrating abdominal injuries: management controversies. Scand J Trauma Resusc Emerg Med [Internet]. 2009;17(1):19. Available from: https://doi.org/10.1186/1757-7241-17-19
2. Wahyuni S, Wahyuni AS, Tarigan R, Syarifah S. Effect of early ambulation to peristaltic activity of abdominal post-operative patients in Medan city hospital, Indonesia. In: IOP Conference Series: Journal of Physics: Conference Series. Institute of Physics Publishing; 2019.
3. The ATLS Subcommittee, Group, Trauma AC of S on, Working TIA. Advanced trauma life support (ATLS®): The ninth edition. J Trauma Acute Care Surg [Internet]. 2013;74(5). Available from: https://journals.lww.com/jtrauma/Fulltext/2013/05000/Advanced_trauma_life_support__ATLS____The_ninth.26.aspx
4. Ozel SK, Kazez A, Akpolat N. Does a fibrin-collagen patch support early anastomotic healing in the colon? An experimental study. Tech Coloproctol. 2006 Oct;10(3):233–6.
5. Hanafiah OA, Abidin T, Ilyas S, Nainggolan M, Syamsudin E. Wound healing activity of binahong (Anredera cordifolia (Ten.) Steenis) leaves extract towards NIH-3T3 fibroblast cells. J Int Dent Med Res. 2019;12(3):854–8.
6. Laila L, Febriyenti F, Salhimi SM, Baie S. Wound healing effect of Haruan (Channa striatus) spray. Int Wound J. 2011;8(5):484–91.
7. Rizal M, Munadziroh E, Kriswandini IL. The increase of VEGF expressions and new blood vessels formation in Wistar rats induced with post-tooth extraction sponge amnion. Dent J (Majalah Kedokt Gigi). 2018;51(1):1–4.
8. Fernandes M, Sridhar MS, Sangwan VS, Rao GN. Amniotic membrane transplantation for ocular surface reconstruction. Cornea. 2005 Aug;24(6):643–53.
9. Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM. Properties of the amniotic membrane for potential use in tissue engineering. Eur Cell Mater. 2008 Apr;15:88–99.
10. Uludag M, Citgez B, Ozkaya O, Yetkin G, Ozcan O, Polat N, et al. Effects of amniotic membrane on the healing of normal and high-risk colonic anastomoses in rats. Int J Colorectal Dis. 2009 Jul;24(7):809–17.
11. Toda A, Okabe M, Yoshida T, Nikaido T. The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. J Pharmacol Sci. 2007 Nov;105(3):215–28.
12. Soylu S, Yildiz C, Bozkurt B, Karakus S, Kurt B, Kurt A. Amniotic Membrane-Coated Polypropylene Mesh for the Repair of Incisional Hernia: An Experimental Study In a Rat Model of Abdominal Wall Defect. Iran Red Crescent Med J. 2018;20(3).
13. Retha R, Sawitri S, Rahmadewi R, Hidayati AN, Listiawan MY, Ervianti E, et al. Combination of Amniotic Membrane Stem Cell Metabolite Product (AMSC-MP) and Vitamin E for Photoaging. Berk Ilmu Kesehat Kulit dan Kelamin. 2020;32(1):1–6.
14. Farhadihosseinabadi B, Farahani M, Tayebi T, Jafari A, Biniazan F, Modaresifar K, et al. Amniotic membrane and its epithelial and mesenchymal stem cells as an appropriate source for skin tissue engineering and regenerative medicine. Artif Cells, Nanomedicine, Biotechnol [Internet]. 2018 Nov 5;46(sup2):431–40. Available from: https://doi.org/10.1080/21691401.2018.1458730
15. Solomon A, Rosenblatt M, Monroy D, Ji Z, Pflugfelder SC, Tseng SCG. Suppression of interleukin 1α and interleukin 1β in human limbal epithelial cells cultured on the amniotic membrane stromal matrix. Br J Ophthalmol. 2001;85(4):444–9.
16. Hao Y, Ma DH-K, Hwang DG, Kim W-S, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. Cornea. 2000;19(3):348–52.
17. Kim JAES, Kim JAEC, Na BKUK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. Exp Eye Res. 2000;70(3):329–37.
18. Forbes J, Fetterolf DE. Dehydrated amniotic membrane allografts for the treatment of chronic wounds: a case series. J Wound Care. 2012 Jun;21(6):290,292,294-296.
19. Zelen CM, Snyder RJ, Serena TE, Li WW. The use of human amnion/chorion membrane in the clinical setting for lower extremity repair: a review. Clin Podiatr Med Surg. 2015 Jan;32(1):135–46.
20. Rieuwpassa IE, Tanca M, Oktawati S, Djais AI,

Cangara MH, Achmad H. Comparison of fibroblast amount on the gingival healing process in the wound applied by platelet-rich plasma (Prp) and chlorhexidine (chx). Indian J Public Heal Res Dev. 2019;10(5):604–9.
21. Oley MC, Islam AA, Hatta M, Hardjo M, Nirmalasari L, Rendy L, et al. Effects of platelet-rich plasma and carbonated hydroxyapatite combination on cranial defect Bone Regeneration: An animal study. Wound Med. 2018;21:12–5.
22. Merchan-Clavellino, A., B. Ribeiro Do Couto, and J. Ramon Alameda-Bailen, *DESCRIPTION OF NEUROPSYCHOLOGICAL EFFECTS IN ADOLESCENCE ASSOCIATED WITH ALCOHOL AND/OR TOBACCO USE.* REVISTA ARGENTINA DE CLINICA PSICOLOGICA, 2019. **28**(4): p. 409-417.
23. Castro-Bouzas, M. and M.A. Talavera-Valverde, *DIFFERENCES BETWEEN THE SEXES OF PERSONS ADMITTED FOR SUICIDAL IDEATION/ATTEMPT AT HIGH RISK IN THE HEALTH AREA OF FERROL. SPAIN.* REVISTA ARGENTINA DE CLINICA PSICOLOGICA, 2019. **28**(4): p. 418-428.
24. Davalos-Picazo, G., et al., *EFFECT OF SYSTEMIC FAMILY THERAPY ON INTERNALIZING AND EXTERNALIZING SYMPTOMS IN ADOLESCENTS.* REVISTA ARGENTINA DE CLINICA PSICOLOGICA, 2019. **28**(4): p. 429-438.
25. Sandra Patricia, G.-B., et al., *EFFECT OF A GROUP INTERVENTION PROGRAM WITH DBT FOR SELF-INJURIOUS BEHAVIORS WITHOUT SUICIDAL INTENTIONALITY.* REVISTA ARGENTINA DE CLINICA PSICOLOGICA, 2019. **28**(4): p. 439-451.