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Effects of Dried Bovine Amniotic Membrane as Prosthetics of Abdominal Fascial Defect Closure Observed by the Expression of Platelet-Derived Growth Factor in *Rattus norvegicus* Wistar Strain

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ABSTRACT

Abdominal wall defect is a condition often occurring after surgery or trauma. Amniotic membrane is believed to contain growth factors including epidermal growth factors (EGF), platelet-derived growth factor (PDGF), and transforming growth factor-beta. This study aimed to analyze differences in PDGF levels in abdominal fascia defects in which closure was performed with and without dried bovine amniotic membrane. This was an experimental research design on experimental animals of 32 *Rattus norvegicus* Wistar strain divided into two groups, control and treatment. PDGF evaluation was measured using the immunohistochemical examination. Measurements were made subjectively by two anatomical pathologists separately with a scoring system. Evaluation of PDGF expression examination was assessed by the intensity and percentage of positive cells. There was no significant difference in PDGF intensity ($p = 0.763$) between the treatment and control groups. Meanwhile, the PDGF extension found a significant difference ($p = 0.005$). PDGF expression was obtained by multiplying the intensity score by extension, and the results obtained were significantly different ($p = 0.008$). PDGF expression in the treatment group was higher than in the control group. Closing the abdominal fascial defect using dried bovine amniotic membranes can increase PDGF expression.

Keywords: effects of the abdominal fascia, dried bovine amniotic membrane, expression of platelet-derived growth factor.

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INTRODUCTION

Stomach surgery is an action involving the abdominal cavity that can be performed with open surgery (1). Abdominal wall defect is a condition often occurring after surgery or trauma. Abdominal wall defect is a condition where there are no components of the abdominal wall. The most important component that can trigger the onset of abdominal defects is the loss of fascia, muscles and the skin with the function to cover the deeper layers (2). In the treatment of abdominal wall defects, there are three essential components: the size of the defect, location of the abdominal wall (upper, middle, and/or lower third; central or lateral), and tissue requirements for reconstruction (partial-thickness defect or full-thickness defect). Another aspect to note is the need to make a tension-free closure to prevent fascial dehiscence. Significant total defects from the midline often require local or distant flaps for abdominal wall reconstruction, coupled with skin grafts or tissue expansion for skin closure. In the development of the Abdominal Wall Reconstruction (AWR) technique, a biological mesh or acellular dermal matrix (ADM) has emerged expected to be a solution in complicated AWR cases. One biological mesh that can be used is the use of amniotic membranes (3).

Various methods to improve wound healing have been applied, one of which involves placing the amniotic membrane in the wound (4). One source of amniotic membrane is from cows. Some of the advantages of bovine amniotic membrane compared to the human amniotic membrane are legality, ethics, and religious problems compared to the use of human amniotic membrane. Bovine amniotic membranes have a higher amount of availability for mass production. Bovine amniotic membrane has many growth factors that will be useful if used as a wound dressing. Bovine amniotic membrane can shorten the reepithelization process so that wound healing can be faster (5). One other consideration in the selection of bovine amniotic membranes is that the use of human amniotic membranes allows for the spread of infections, such as hepatitis, syphilis, tuberculosis, and AIDS. (6).

Wound healing is a complex cellular and biochemical cascade leading to restitution of the integrity and function of a tissue (7). Under normal circumstances, the wound healing process follows a predictable pattern and can be divided into several phases. The entire wound healing process involves a series of complex events starting at the time of the injury and can continue for months to years (8). Several types of growth factors and cytokines are released in this process (9). One

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growth factor that has an essential role in the process of wound healing is platelet-derived growth factor (PDGF) (10).

Amniotic membrane is believed to contain growth factors, including epidermal growth factors (EGF), PDGF, and transforming growth factor-beta. These growth factors are present in granules in concentrated platelet-rich plasma (PRP) (11,12). PDGF is a growth factor first appearing in the wound healing process, which plays a significant role in wound healing and is a major player in the wound healing process. The initial function of PDGF is to stimulate the formation and proliferation of fibroblasts. The next function is to induce myofibroblast phenotype. Meanwhile, EGF functions in the re-epithelialization process. In addition to the amniotic membrane, PDGF is also believed to be produced by human body tissues (13,14). Hence, this research needs to be conducted to determine whether there are differences in PDGF levels in the abdominal fascial defect in which closure was performed with or without dried bovine amniotic membranes.

METHODS

An experimental study using rat species of *Rattus norvegicus* Wistar strain selected and taken from the experimental animal unit of the Pharmacology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The research subjects were selected 32 Wistar rats aged between 12-14 weeks, with the bodyweight of between 250-300 grams, divided into two groups. The first group consisted of 16 rats as the control group, and the second group consisted of 16 rats as the treatment group. Study samples were selected from the same place (Farma Veterinary Center) and given the same food. Until the observation time was complete, none of the study samples dropped out.

Sample flap over the fascia with a size of 5x3 cm and manipulation of the abdominal wall in the form of making a defect in the fascia of 2x2 cm were performed in both groups. Direct defect closure with a skin flap, which was closed with interrupted sutures using 3-0 nylon, was done in the first group

as a control group. Meanwhile, bovine amnion was placed under the skin after manipulating the abdominal wall in the second group as a treatment group. Sample rats were sacrificed on day 21 by injecting pentobarbital 60-100 mg/kg intra-muscular body weight, and the effects of different PDGF levels were examined by taking skin flap tissue containing wounds for anatomical pathology examination.

PDGF was examined by taking full thickness from the wound tissue and checking it by immunohistochemical staining with anti-PDGF BB antibodies. PDGF BB can represent all the receptors in the wound tissue (α and β). The level of PDGF expression was measured subjectively by 2 Anatomic Pathologists separately with a scoring system. Measurement of expression from PDGF was done by assessing the intensity and percentage of positive cells to evaluate immunoreactivity. The intensity of PDGF was expressed in 0-3 scores regarding the intensity of the colored PDGF. Extensions from stained cells were expressed in grades 0 for 1-9%, 0.5 for 10-50%, and 1 for extensions higher than 50%. The scores were generated by multiplying the intensity value with the extension of the colored PDGF.

Kappa statistics were used to assess the consistency of 2 anatomic pathologists in assessing the intensity of PDGF. Data obtained in the study were then recorded and analyzed with the independent t-test.

RESULTS

The basic data of the research sample are illustrated in Table 1. The results of the comparison test on the age data using the Mann-Whitney test and weight data using the T-test comparison test showed sig.2 tailed for $p = 0.491$ for the age data and $p = 0.256$ for weight data ($p > 0.05$). This means that there were no significant differences in the rat's age and weight between the two groups. These results state the data were still homogeneous. Thus, there was no bias from differences in body weight and age of Wistar rats.

Table 1: Comparison of mean age and weight between groups

Variables	Groups		P
	Control	Treatment	
Age (weeks)*	12.69±0.793	12.88±0.806	0.491
Body weight (grams)**	268.31±12.520	273.44±11.454	0.236

Note: data are presented as Mean ± SD; Shapiro-Wilk normality test; *) Mann-Whitney U test; **) T-test comparison test

The sacrifice of rats using phenobarbital was carried out on the 21st day. Then the specimen collection was taken and fixed with formalin. Measurement of expression from PDGF was performed by assessing the intensity and percentage of positive cells to evaluate immunoreactivity. The intensity of PDGF was expressed in 0-3 regarding the intensity of the

colored PDGF (Figure 1). Extensions from stained cells were expressed in grades 0 for 1-9%, 0.5 for 10-50%, and 1 for extensions higher than 50%. The score was generated by multiplying the intensity value with the extension of the colored PDGF (Figure 2).

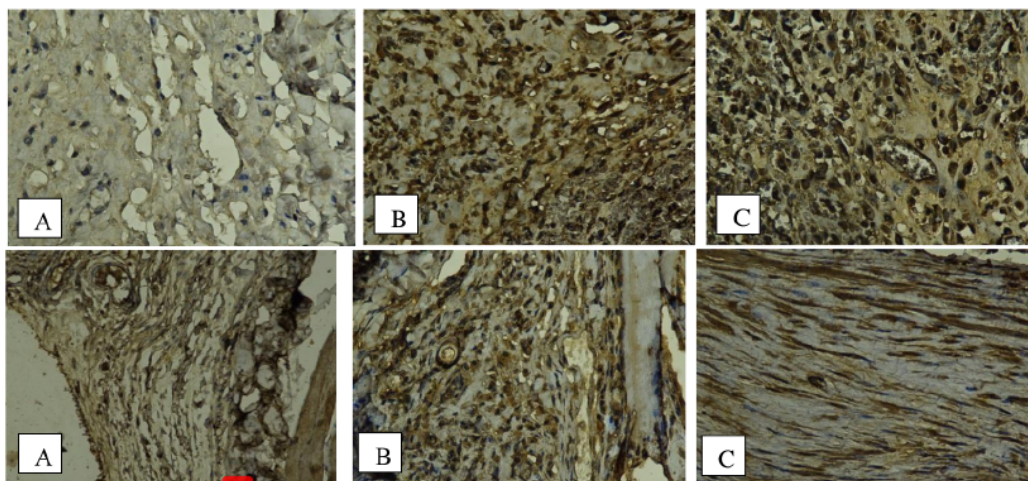


Figure 1: PDGF intensity A. Score 1; B. Score 2; C. Score 3

Figure 2: PDGF extension A. Score 0; B. Score 0.5; C. Score 1

Table 2 shows the results of subjective measurements of intensity, extension, and expression of PDGF between treatment and control groups. Intensity scores were found in the treatment group with the highest score of 2 of 9 (56.3%) samples and in the control group of 10 (62.5%) samples, which had a score of 2 ($p = 0.763$). The extension score was found in the treatment group with the highest score 1 with a total sample of 9 (56.3%), while the control group had the highest score with score 1 with 11 (68.8%) ($p = 0.005$). After the PDGF intensity and extension values are obtained, these

two values multiply to get the PDGF expression. From the result of the comparison test using the T-test, it was found that there were significant differences in the expression of PDGF between the treatment and control groups with $p = 0.008$ ($p < 0.05$). This indicates that statistically, the administration of the amniotic membrane to the abdominal defect's closure can increase PDGF expression. PDGF expression scores in the treatment and control groups can be seen in Figure 3.

Table 2: Subjective measurements of PDGF intensity, extension, and expression between treatment and control groups.

Measurement	Groups		p
	Treatment	Control	
Intensity			
0	-	-	
1	2 (12.5%)	2 (12.5%)	0.763*
2	9 (56.3%)	10 (62.5%)	
3	5 (31.3%)	4 (25%)	
Extension			
0	-	3 (18.8%)	0.005*
0.5	7 (43.8%)	11 (68.8%)	
1	9 (56.3%)	2 (12.5%)	
Expression			
0	-	3 (18.8%)	
0.5	2 (12.5%)	1 (6.3%)	0.008**
1	2 (12.5%)	6 (37.5%)	
1.5	3 (18.8%)	4 (25%)	
2	7 (43.8%)	2 (12.5%)	
3	2 (12.5%)	-	

Note: *) Mann-Whitney test; **) T-test

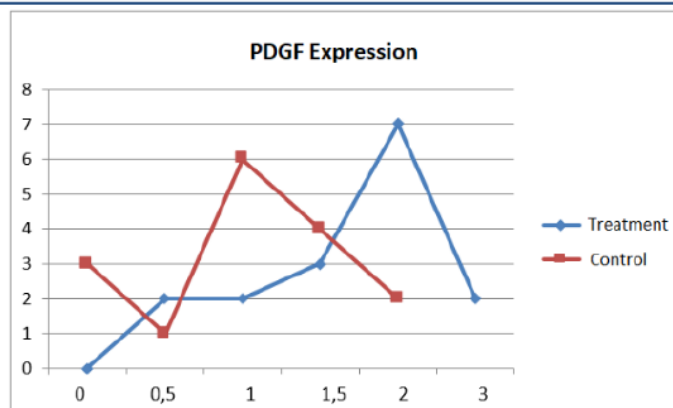


Figure 3: PDGF expression scores in the treatment and control groups

DISCUSSION

This study proves that there are significant differences in PDGF expression between the treatment group and the control group. This means that in the treatment group, the PDGF expression was higher than that of the control group. Clinically, it means that groups with higher PDGF expression have faster healing. According to Werner's research, the level of PDGF in wounds that do not heal is lower (13).

Wound healing is a complex process that begins with the disruption of tissue integrity. In reasonable condition, the wound can heal by itself, but how fast it is can be affected by many factors (15). Several types of growth factors are found in the process of wound healing with an important role in this process. Several types of growth factors have been approved for use as a treatment in humans. The growth factors are PDGF, FGF-2, IGF, and KGF (16). PDGF is the first growth factor appearing in the process of wound healing. This shows that PDGF plays a vital role in the process. PDGF acts as a chemotactic factor. The role of PDGF here is to attract neutrophil cells, monocytes, fibroblasts, and smooth muscle cells to the wound site. In addition, PDGF also plays a role in the proliferation of fibroblasts and the production of extracellular matrix (13).

PDGF can influence the difference in wound healing speed because PDGF has an important role in wound healing. The initial function of PDGF is to stimulate the formation and proliferation of fibroblasts. The existence of PDGF will accelerate wound healing. The next function is to induce myofibroblast phenotype. This hypothesis is supported by evidence that neutralizing PDGF antibodies in wound fluid will cause a 45% reduction in the mitotic process of fibroblasts cultured from wound fluid (13).

This study is also in accordance with a previous study applying a single dose of PDGF-BB (200pm) and observing the wound healing process. From the observations, in the initial three weeks, the wound healing power of rats given by PDGF-BB was 150-170% from control rats. This increased strength acceleration occurs on days 4-6 in the first two weeks. On the 89th day, the healing power of the wounds of both rats had the same strength. The healing power of the two wounds was around 90% of normal tissue (17).

PDGF has the most significant role before day 7 of the wound

healing process. PDGF plays a role in accelerating the initial inflammatory response, which will accelerate the wound healing process. In addition, PDGF is a major player in the process of wound healing (18). Thus, the use of amniotic membrane itself is the same as applying a single dose of PDGF into the wound area. This study shows that the wound area given PDGF had a higher expression of PDGF. We suspect that this amniotic membrane stimulates the expression of PDGF and several other growth factors.

CONCLUSION

There was a significant increase in PDGF expression in abdominal fascia defects in which the closure was performed using an amniotic membrane compared to fascia defects that were closed using only a skin flap. Hence the amniotic membrane can be used as a local material that can be applied to accelerate the wound healing process in the reconstruction of abdominal wall defects. Further research is needed by evaluating PDGF at different times, such as on the 7th and 14th days. Moreover, further research needs to be done by reducing the bias and measurement of more objective PDGF expressions.

ETHICAL CLEARANCE

The Ethics Committee approved this research before this research was conducted. Moreover, all experimental animals were treated in accordance with the rules of the Animal Care and Use Committee of Universitas Airlangga, Surabaya.

CONFLICTS OF INTEREST

The authors state that there is no conflict of interest in this study.

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CONTRIBUTION OF AUTHORS

All authors have contributed to all processes in this research, preparation, review, and approval of this research.

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