Sys Rev Pharm 2020;11(5):574-577
A multifaceted review journal in the field of pharmacy

Effect of Bovine Dried Amniotic Membranes as Prosthesis of Abdominal Fascial Defect Closure Reviewed from The Level of Density Collagen Type I and III in *Rattus novergicus* Wistar Strain

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ABSTRACT

Collagen types I and III is an important component of abdominal fascia and aponeurosis. Dried amniotic membrane is rich in the extracellular matrix, dominated by types I and III collagen, which has the potential to improve the wound healing process. This study aimed to analyze the differences in density of types I and III collagen in abdominal fascia defects closed with and without bovine dried amniotic membranes. This was an experimental research design using Rattus norvegicus divided into two groups. The first group was directly covered with a skin flap closure, while the second group used bovine dried amniotic membrane. Evaluation of type I and III collagen density was assessed using an immunohistochemical examination and a collagen density score, the Pathological Visual Score. In the evaluation of type I collagen density, the score in the control group was 1 (68.8%), 0 (25%), and 3 (6.3%). Whereas in the treatment group was a score of 2 (50%), a score of 1 (43.8%), and a score of 0 (6.3%). The density of type III collagen showed that the scores in the control group were a score of 1 (50%), a score of 0 (31.3%), and a score of 2 (18.8%). Meanwhile, the treatment group obtained a score of 2 (68.8%), a score of 1 (31.3%), and a score of 0 (0%). There was an increase in the density of collagen types I and III in the abdominal fascial defect reconstructed using bovine dried amniotic membrane.

Keywords: abdominal fascial defect, bovine dried amnion membrane, collagen type I density, collagen type III density.

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INTRODUCTION

The procedure involving the abdominal cavity is an open surgery (1). The fascia is one of the important structures in the anterior abdominal wall. The anterior abdominal wall has been the subject of numerous studies. The myoaponeurotic lining of the anterior abdominal wall plays a functional and aesthetic role. In linea alba, the region where the abdominal wall consists only of aponeurosis without any muscle covering is exposed to full pressure from the intraabdominal area and becomes a potential problem if there is a defect (2).

As an important part of abdominal fascia and aponeurosis, including linea alba, collagen has an important role as a structure providing support and resistance to the abdominal wall against intraabdominal pressure. Type I collagen is the most abundant component, which is a major component of aponeurosis, tendons, and mature scar tissue. It plays a major role in fascial resistance to strain forces. Type III collagen, formerly known as reticular fibers, functions to support structures with the ability to develop. Type III collagen also increases in number during the initial phase of wound healing (3).

Previous studies have shown that patients with abdominal wall hernias have lower levels of collagen types I and III compared to the control group without hernias. The analysis of collagen types I and III gene expression in abdominal wall fascia in obese patients shows that collagen types I and III are lower than in patients without obesity (4).

Based on socio-economic considerations and the clinical impact of wounds, innovative solutions have been developed for many years, especially the use of extraembryonic stem cell derivatives from the placenta, chorion and amniotic membrane, which have been the subject of numerous studies and tissue engineering. Amniotic membrane has been widely used as a synthetic prosthesis [1]. It has the advantage of having a low antigenicity, antimicrobial, and ability to reduce exudate and adhesion, accelerate epithelialization, reduce local pain, and as a material for tissue growth. The basement membrane portion of the amniotic membrane is rich in an extracellular matrix dominated by collagen types I, III, and fibronectin (5).

The previous study has shown that the use of amniotic membranes in wounds on rat skin can improve wound healing processes and increase the amount of collagen type III in the proliferation phase and collagen type I in the wound maturation phase (6). The use of amniotic membranes in fascial wounds or defects and their benefits in increasing the amount of collagen, especially types I and III, has never been done before. This study aimed to analyze differences in the density of collagen types I and III in abdominal fascia defects covered with and without bovine dried amniotic membrane [2].

METHODS

An experimental study using a randomized control trial design conducted on experimental animals of Wistar strain *Rattus novergicus* aged around 12-16 weeks, with a range of body weight between 250-300 grams. There were two groups, a control group and the treatment group. Observations were made on the 21st day to determine changes in the research variables during the proliferation and maturation phases of the wound. Before being sacrificed, repeated body measurements were taken for each animal. Experimental rats were adapted for seven days, then randomized by permuted block randomization, and the samples were divided into control groups and treatment groups. After a defect was made in the abdominal fascia, the control group was covered with a skin flap, while the treatment group was added with bovine dried amniotic membrane. After the maturation phase (21 days), an immunohistochemical examination was performed to determine the level of collagen density types I and III using collagen I and III polyclonal antibodies. The assessment of collagen density uses the Pathological Visual Score are as follows:

Score 0: No collagen fibers found in the wound area (0%)
Score 1: Density of collagen fibers in low wound area (<10%)
Score 2: Density of collagen fibers in moderate injury area (10-50%)
Score 3: Density of collagen fibers in tightly wound area (>50%)

RESULTS

The study was conducted on 32 male *Rattus novergicus* Wistar strain with age between 12-16 weeks with a body weight between 250-300 grams. Sixteen rats were involved in the control group, and 16 more in the treatment group. The basic data of the research sample are illustrated in Table 1. Data on body weight and age of the samples were normally distributed with a P-value >0.05.

Table 1. Characteristics of samples

Characteristics	Groups		p
	Control	Treatment	
Age (weeks)	14.13	13.63	0.236
Weight (grams)	272.50	269.37	0.590

Type I Collagen Density Test

In the type I collagen density score data, in the treatment group, the most score found was score 2 in 8 samples (50%), followed by score 1 in 7 samples (43.8%) and score 0 in 1 sample (6.3%), with an average score of 1.44. Meanwhile, the highest score in the control group was score 1 in 11 samples (68.8%), followed by score 0 in 4 samples (25%) and score 2 in 1 sample (6.3%), with an average score of 0.81. In this study, the difference in type I collagen density test illustrated through the type I collagen density score was performed using the Mann-Whitney U test because the type I collagen density score data were ordinal data. Mann-Whitney U test results obtained p <.05 (0.006). It can be concluded that differences in type I collagen density in the control group compared with the treatment group were statistically significant.

Table 2. Type I collagen density scores

Groups	Score 0	Score 1	Score 2	Mean	p
Treatment	1 (6.3%)	7 (43.8%)	8 (50%)	1.44	0.006
Control	4 (25%)	11 (68.8)	1 (6.3%)	0.81	

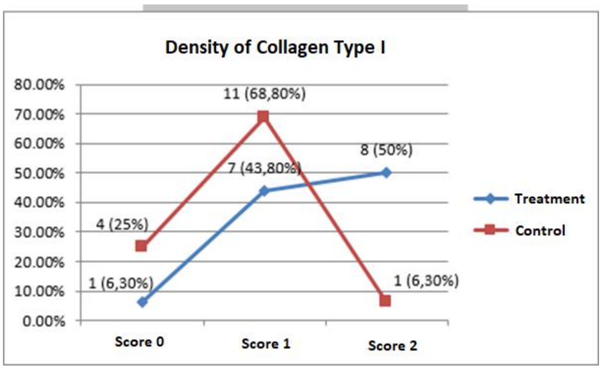

Figure 1. Density of Collagen Type I

Type III Collagen Density Test

In the type III collagen density score data, in the treatment group, the most score was score 2 in 11 samples (68.8%), followed by score 1 in 5 samples (31.3%) and score 0 in 0 samples (0%), with an average score of 1.69. Meanwhile, the highest score in the control group was score 1 in 8 samples (50%), followed by score 0 in 5 samples (31.3%) and score 2 in 3 samples (18.8%), with an average score of 0.88. In this study, the difference in type III collagen density test illustrated through the type III collagen density score, performed using the Mann-Whitney U test because the type III collagen density score data were ordinal data. Mann-Whitney U test results showed p <0.05 (0.002). It can be concluded that differences in type III collagen density in the control group compared with the treatment group were statistically significant.

Table 3. Type III collagen density scores

Groups	Score 0	Score 1	Score 2	Mean	p
Treatment	0 (0%)	5 (31.3%)	11 (68.8)	1.69	0.002
Control	5 (31.3%)	8 (50%)	3 (18.8 %)	0.88	

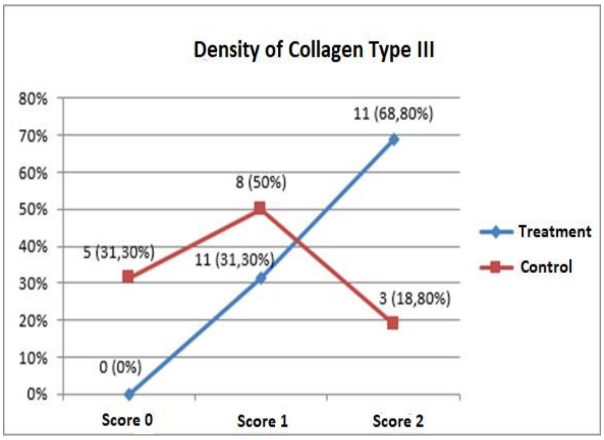

Figure 2. Density of Collagen Type III

DISCUSSION

The results of this study showed a statistically significant increase in density of collagen types I and III in the treatment group compared to the control group. These results indicate that the nutritional status of experimental animals did not influence the final results for both collagen types I and III density scores, in which case there was no significant difference in weight changes before and after treatment. Also, there were no side effects, such as hematoma, hernia, infection and cardiorespiratory disorders in both the control and treatment groups. The whole process of wound healing involves a series of complex events which can take a long time (7). In normal conditions, the wound can heal by itself, but how fast it is can be affected by many factors (8). Various methods to improve wound healing have been applied, one of which involves placing the amniotic membrane in the wound (9). This study proves that the use of amniotic membrane as a prosthesis to close the abdominal fascial defect can increase the density of collagen types I and III in the fascial defect [3]. The epithelium and stroma of the amniotic membrane are abundant in the expression of several growth factors, including TGF-α, epidermal growth factors (EGF), keratinocyte growth factors (KGF), hepatocyte growth factors (HGF), and basic fibroblast growth factors (bFGF) (10). These growth factors are present in granules in concentrated platelet-rich plasma (PRP) (11,12). These growth factors will stimulate an increase in excess extracellular matrix deposits in the maturation phase of wound healing. Fibronectin and collagen type III are the initial matrix formed. Meanwhile, glycosaminoglycans and proteoglycans will be formed in the next matrix. The final matrix formed in the maturation phase is type I collagen, largely replacing the amount of type III collagen formed during the initial matrix formation [4].

Amniotic membrane can accelerate the process of formation of collagen types I and III (13) evidenced from the control group with the highest scores of 0 and 1, compared to the treatment group with the highest scores of 1 and 2. These results were obtained from collagen types I and III. It can be concluded that with the amniotic membrane, the scoring process of forming collagen types I and III was quickly passed from score 0 to score 3. The presence of amniotic membrane contact with the wound base is associated with the ability to recover using a fast bovine amnion membrane. The amniotic membrane consists of cube-shaped, enlarged epithelial cells and mesenchymal connective tissue. Epithelial cells migrate along the amniotic membrane and form the edge of the epidermis. Although bovine amniotic membrane epithelial cells are destroyed through various sterilization and storage processes, the connective tissue in them remains present. The connective tissue of the amniotic membrane contains laminin, fibronectin, collagen types I and III, which are the main components of the dermal basal membrane. Thus, the rapid and complete epithelialization process is the result of wound closure with an amniotic membrane (14).

This study generally shows that the application of amniotic membranes can stimulate the process of collagenization. This is evidenced by an increase in the number of collagen types I and III, acting as the main structure of the new extracellular matrix of wound tissue and experiencing a relatively increased amount on the 21st day. However, how its development until then can grow new fascial tissue still needs to be further investigated.

CONCLUSION

The use of bovine dried amniotic membrane can increase the density of both type I and 3 collagen in abdominal fascial defects of *Rattus novergicus* Wistar strain after reconstruction and is statistically significant. Bovine dried amniotic membrane can be used as a local material applied to improve the extracellular matrix structure of wound tissue in the abdominal fascia of rats.

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