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The effect of free peritoneal patch on fibroblast count and density of collagen on primary colon anastomosis in intraperitoneal infection in New Zealand White Rabbit model

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ABSTRACT

Background: In intraperitoneal infection or peritonitis, intestinal resection and anastomosis might result in severe morbidity and intestinal anastomosis leakage have been documented to be fatal. We aim to examined the effect of free peritoneal patch on fibroblast count and density of collagen on primary colon anastomosis in intraperitoneal infection.
Methods: This is an experimental study with randomized control trial design using New Zealand White Rabbit. We collected sample's characteristic data (e.g., age, weight before and after intervention), fibroblast and collagen levels.
Results: The mean of fibroblast level in treatment group was higher than the mean of fibroblast level in control group but there was no significant difference between both group (P value=0.202). The medial collagen level in treatment group was 2, higher than the medial collagen level in control group that was 1 and there was significant difference between both group (P value = 0.010).
Conclusion: The free peritoneal patch on colon anastomosis in intraperitoneal infection can increase fibroblasts and collagen levels compared to those without free peritoneal patch.

Keywords: free peritoneal patch, fibroblast count, collagen density, primary colon anastomosis, intraperitoneal infection.
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BACKGROUND

Intestinal anastomosis is a procedure that is often performed to connect the intestine after resection of the intestine. When conducted during a peritonitis or intraperitoneal infection, intestinal resection and anastomosis might result in severe morbidity. In cases of intraperitoneal infection or peritonitis, intestinal anastomosis leakage have been documented to be fatal, with an incidence rate of 6-22%.[1,2]

Treatment that can be given in the condition of a colon anastomosis leakage includes resection and re-anastomosis. Various risk factors associated with anastomosis leakage including local and systemic factors; both contribute to poor healing and anastomotic failure. As the result, various surgical techniques and high-quality surgical suture materials, parenteral nutrition, the use of prophylactic antibiotics and various protective biomaterials have been developed. Patch made from living tissue is referred as biological dressings and can serve as a regenerative framework to facilitate remodeling and deposition of new collagen.[3]

Biomaterials used as biological dressings can reduce the risk of body rejection when using patch in the form of structures that already exist in the body. Several patches can be used as biological dressings, such as serosal and free peritoneal patches. The free peritoneal patch works by converting into a scaffold matrix that provides fibroblasts a place to grow and produce collagen, which is required for tissue healing to treat anastomosis leakage that causes peritoneal infection.[4] The New Zealand White Rabbit was used for the reason that these experimental animals have similarities with humans in terms of the anatomical structure of the abdomen, liver, and intestinal system. In addition, the digestive system of New Zealand rabbits is physiologically and biochemically similar to humans.

One of the signs of anastomosis healing process is the formation of a connective tissue matrix. Fibroblasts produce the formation of the connective tissue matrix. The components of the matrix are type I and type III collagen. In addition, an increase in vascular endothelial growth factor (VEGF) also affects the increase in angiogenesis which will increase the number of fibroblasts that lead to an increase in collagen production which is important in formation of anastomotic tissue. Platelet-derived growth factor (PDGF) also plays a role in stimulating and producing mesenchymal cells including fibroblasts. Healthy growth of peritoneum in free peritoneal patch can also help the process of closing the defect so that the defect can close properly. The

peritoneum is a reactive tissue and is not thrombogenic, so the peritoneum can support tissue growth and the formation of new blood vessels.[5] In addition, peritoneal mesothelium cells have stem cell-like properties, namely plasticity, which means that when these cells are placed in the right conditions, they can develop into other mesenchymal cells.[6] In this study, we observe the fibroblast count and the density of collagen type I and type III as markers of secondary wound healing that are important in the treatment of intraperitoneal wounds.[7] If this study found that the free peritoneal patch can increase the number of fibroblasts and collagen type I and type III, then the free peritoneal patch can be considered an option as a dressing alternative for colon anastomosis.

MATERIALS AND METHODS

Study design and participants

This is an experimental study with randomized control trial design using New Zealand White Rabbit. At the beginning of the study, the sample was homogenized before conducting randomization for the case group and the control group. Measurements of the variable were conducted at the end of the study.

Data collection

Age, weight, length of the procedure, fibroblast count and collagen density of the rabbits were all documented. To ensure the validity of the variable, the test tools and materials were selected that have high sensitivity and specificity, consistent and can be accounted for.

Statistical analysis

The data were analyzed using the SPSS 23.0 (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). The variables were analyzed by normality test using the Saphiro Wilk test. If the data is normally distributed ($p>0.05$), it was continued with statistical t-independent test (Independent t-test) to see the difference between the two groups. If the data were not normally distributed ($P < 0.05$), it was continued with the Mann-Whitney U statistic test to see the difference between the two groups. P value of less than 0.05 was considered as statistically significant.

RESULTS

Description of research data

This study is an experimental study with randomized control trial design to examine the effect of free peritoneal patch on fibroblast count and density of collagen on primary colon anastomosis in intraperitoneal infection in New Zealand White Rabbit. In this study the authors used New Zealand White Rabbit as experimental animals. At the beginning of the study, the research sample will be homogenized before randomization for the case group and control group will be carried out with the aim to reduce the research bias. Variable measurements were carried out at the end of the study. The research sample was New Zealand White Rabbit as the experimental rabbit, chosen from the experimental animal unit of the biochemistry laboratory, Faculty of Medicine, Airlangga University, aged 6 to 9 months and weighed between 1,900 grams and 2,500 grams.

Characteristics of research samples

In this study, the mean age of the control sample was 7.53 months with standard

Table 1. Characteristics of research samples.

Characteristics	Group		P value
	Control	Treatment	
Age (month)			
Mean	7.53	7.6	
Standard Deviation	0.61	0.8	0.806
Median	7	8	
Weight before intervention (gram)			
Mean	2120	2163	
Standard Deviation	101.8	122.7	0.267
Median	2140	2160	
Weight after intervention (gram)			
Mean	2030	2068	
Standard Deviation	102	125.5	0.436
Median	2010	2060	

Table 2. The results of fibroblast count in control group.

No	Sample Code	Fibroblast					Mean	SD
		LP1	LP2	LP3	LP4	LP5		
1	K1	28	29	21	26	34	27.6	4.2
2	K2	23	22	28	29	31	26.6	3.5
3	K3	28	23	23	29	30	26.6	3.0
4	K4	24	20	24	29	31	25.6	3.9
5	K5	43	40	34	35	38	38.0	3.3
6	K6	36	33	30	29	35	32.6	2.7
7	K7	26	24	22	27	22	24.2	2.0
8	K8	32	36	32	28	29	31.4	2.8
9	K9	25	28	28	21	29	26.2	2.9
10	K10	30	31	23	30	34	29.6	3.6
11	K11	34	32	27	27	24	28.8	3.7
12	K12	24	27	23	28	31	26.6	2.9
13	K13	22	24	28	20	30	24.8	3.7
14	K14	34	44	42	32	30	36.4	5.6
15	K15	33	39	26	27	30	31.0	4.7

Table 3. The results of fibroblast count in treatment group.

No	Sample Code	Fibroblast					Mean	SD
		LP1	LP2	LP3	LP4	LP5		
1	PK1	22	26	28	21	30	25.4	3.4
2	PK2	33	36	30	29	30	31.6	2.6
3	PK3	27	29	33	27	27	28.6	2.3
4	PK4	30	26	25	27	32	28.0	2.6
5	PK5	33	20	28	27	32	28.0	4.6
6	PK6	36	38	22	27	20	28.6	7.3
7	PK7	30	31	27	37	39	32.8	4.5
8	PK8	40	42	30	36	33	36.2	4.4
9	PK9	24	26	30	28	20	25.6	3.4
10	PK10	29	40	41	33	36	35.8	4.4
11	PK11	50	44	48	39	37	43.6	5.0
12	PK12	32	24	28	35	36	31.0	4.5
13	PK13	44	40	43	44	38	41.8	2.4
14	PK14	23	20	22	28	30	24.6	3.8
15	PK15	43	38	42	35	35	38.6	3.4

Table 4. The results of collagen density in control group.

No	Sample Code	Grade of Collagen
1	K1	1
2	K2	2
3	K3	1
4	K4	1
5	K5	1
6	K6	1
7	K7	1
8	K8	1
9	K9	1
10	K10	1
11	K11	1
12	K12	1
13	K13	1
14	K14	1
15	K15	1

Table 5. The results of collagen density in treatment group.

No	Sample Code	Grade of Collagen
1	PK1	3
2	PK2	2
3	PK3	2
4	PK4	3
5	PK5	2
6	PK6	1
7	PK7	1
8	PK8	2
9	PK9	2
10	PK10	2
11	PK11	1
12	PK12	1
13	PK13	1
14	PK14	1
15	PK15	2

deviation of 0.61 months and median value of 7 months. The mean age of the treatment sample was 7.6 months with standard deviation of 0.8 months and median value of 8 months. The results of our analysis showed that there was no significant difference between the age of the control group and the treatment group with P value = 0.806.

The mean weight before the intervention on the control sample of 2.120 grams with a standard deviation of 101.8 grams and a median value of 2.140 grams. The mean weight before the intervention on the treatment sample of 2.163 grams with a standard deviation of 122.7 grams and a median value of 2.160 grams. Our analysis showed no significant difference between the weight before intervention of the control group and the treatment group with P value = 0.267.

The mean weight after the intervention on the control sample of 2,030 grams with standard deviation of 102 grams and median value of 2,010 grams. The mean weight after the intervention on the treatment sample of 2,068 grams with standard deviation of 125,5 grams and median value of 2,060 grams. Our analysis showed no significant difference between the weight after intervention of the control group and the treatment group with P value = 0.436. Sample characteristics are shown in Table 1.

The results of fibroblast count in control group

Researchers measured the levels of fibroblasts in the specimens using histopathological approach. Fibroblast levels were observed by noting the distribution of fibroblast cells in 5 different microscopic fields of view. The fibroblast level in the sample was determined by calculating the mean of the fibroblast count in 5 fields of view. The highest mean fibroblast level in control group was 38.0 with standard deviation of 3.3, while the lowest was 24.2 with standard deviation of 2.0 (range 24.2 – 38.0; 15.8). In general, the fibroblast level was 29.1. The result of fibroblast levels in control group are shown in Table 2.

The results of fibroblast count in treatment group

Similar to control group, the levels of fibroblasts in the specimens using histopathological approach. Fibroblast levels in the treatment group were observed by noting the distribution of fibroblast cells in 5 different microscopic fields of view. The highest mean fibroblast level in treatment group was 43.6 with standard deviation of 5.0 while the lowest was 24.6 with standard deviation of 3.0 (range 24.6 – 43.6; 21.0). In general, the fibroblast level was 32.0. The result of fibroblast levels in treatment group are shown in Table 3.

The results of collagen density in control group

Researchers measured the levels of collagen in the specimens using histopathological approach.

Histologically, simple staining cannot differentiate between type 1 and type 3 collagen. Therefore, the calculation will be carried out on collagen in general. Collagen levels will be observed by noting the distribution of collagen cells in 5 different microscopic fields of view. It was

Table 6. Comparison of fibroblast and collagen level.

Group	Median fibroblast	P value	Median collagen	P value
Control Group	29.1		1	
		0.202		0.010*
Treatment Group	32.0		2	

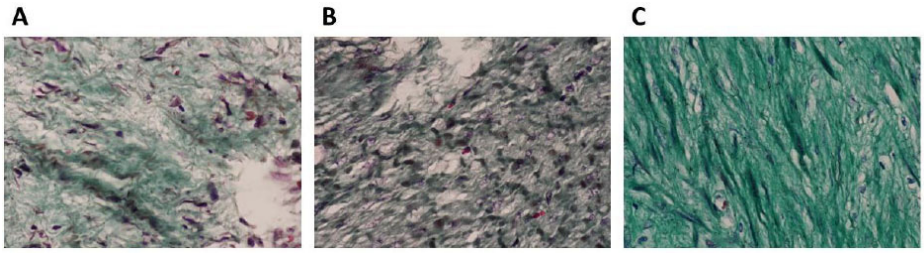

Figure 1. Histopathological view of collagen (under 400x magnification). (A) grade 1; (B) grade 2; (C) grade 3.

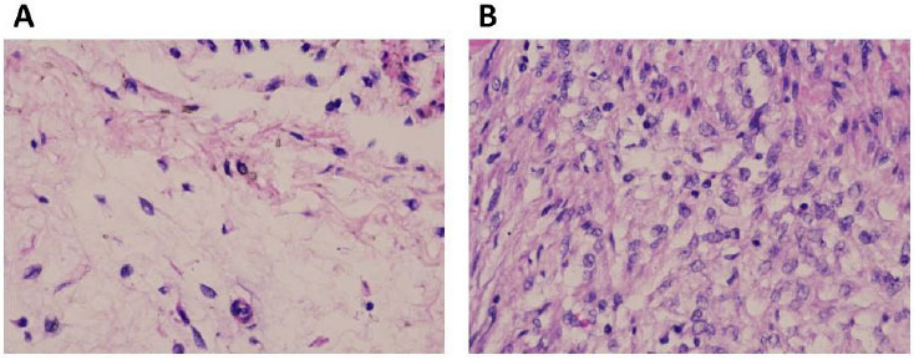

Figure 2. Histopathological view of fibroblast (under 400x magnification). (A) low density; (B) high density.

found that of 15 control samples, almost all had a collagen distribution of degree 1 (14/15; 93.3%). There was only one patient with a degree of collagen distribution of 1 (1/15; 6.7%). The result of density of collagen in control group is shown in table 4.

The results of collagen density in treatment group

Collagen levels were be observed by noting the distribution of collagen cells in 5 different microscopic fields of view. There were 6 samples with collagen grade 1, 7 samples with collagen grade 2, and 2 samples with collagen grade 3. In the treatment group, sample PK6, PK7, PK11, PK12, PK13, and PK14 have collagen grade 1. Sample PK2, PK3, PK5, PK8, PK9, PK10, and PK15 have collagen grade 2. Samples PK1 and PK4 have the highest collagen grade 3. The median of collagen grade in treatment group is collagen grade 2. The result of density of collagen in treatment group are shown in table 5.

Comparison of fibroblast and collagen level

Researchers compared the fibroblast level between the control group and treatment group. In this study, the average of fibroblasts in the control group was 29.1, but the average of fibroblasts in the treatment group was 32.0. Our analysis showed no significant difference between the mean fibroblast counts in the control group and the treatment group with P value = 0.202.

The median collagen level in the control group had an average of 1, while the median level of collagen in the treatment group had an average of 2. Our analysis showed a significant difference between the mean collagen level in the control group and the treatment group with P value = 0.010.

DISCUSSION

In this study, we found that there is no significant difference between the characteristics of the research sample both in the control group and the treatment group. This suggests that the rabbit sample in this study is homogeneous. This is crucial because an inhomogeneous sample can potentially cause a research bias that can affect research results.

In this study we examined fibroblast levels in both study groups. In this study, it was found that the mean value of fibroblast level in the treatment group was higher than the mean value of fibroblast level in the control group. These results support our theory that fibroblast level was higher in the treatment group that received free peritoneal patches. The result of this study is also supported by other studies which state that the use of a peritoneal patch is a procedure option that provides advantages in cases of large bowel anastomosis. Fibroblast itself is a factor that plays an important role in the wound healing process.

In addition to fibroblast, collagen is also a component that plays an important role in the wound healing process. This study found that the mean value of collagen levels in the treatment group was higher than the mean value of collagen levels in the control group. These results support our theory that collagen level was higher in the treatment group that received free peritoneal patches. The result of this study is also supported by other studies which state that the use of a peritoneal patch is a procedure option that provides advantages in cases of large bowel anastomosis. The result of this study is also supported by other studies which state that the use of peritoneal patches can affect the expression of collagen level in surgical wounds.

Thing to keep in mind is that the treatment in this trial was given to rabbits that were suffering from an intraperitoneal infection. Intraperitoneal infection is a condition the surgeon considers when deciding an intestinal anastomosis procedure. This is because primary suturing in intraperitoneal infection situation can raise the incidence of problems such as suture breakdown, which can impact the result. According to earlier research, wound dehiscence at the site of intestinal resection and anastomosis is a risk factor for septic peritonitis.[8,9] Preoperative peritonitis was a significant risk factor for the development of anastomotic leak

in this study. Bacteria and inflammatory cells produce collagenase, which reduces the intestinal wall's collagen content and weakens anastomosis strength.[10-12] A previous experimental study examining intestinal anastomoses performed on 181 dogs found that 30% of dogs had fecal leakage in the group with intentional peritonitis, compared with only 7.4% in the group without fecal contamination.[11] In a clinical study, dogs and cats without preoperative peritonitis were found to have a leakage rate of 10%, whereas dogs and cats with peritonitis had a leakage rate of 26.6%.[13] Because of the high risk of leakage, many surgeons avoid direct bowel anastomosis procedures in patients with generalized peritonitis and instead choose to fecal diversion with stoma opening. Previous studies have shown that the use of collagen fleece or collagen patch is closely associated with increased granulation tissue, increased expression of mRNA level of collagen type I and type III, and higher level of VEGF when compared to the use of primary suture technique alone.[9]

The data presented in this study demonstrate the potential of using free peritoneal patch as an effective method to prevent leakage of colon anastomosis in peritonitis. Although this method still debates among clinicians, this method can be an alternative. This method can increase the expression of collagen and fibroblasts, which are 2 important components in the wound healing process and tissue strength. With increased levels of these two substances, it is hoped that the strength of the tissue at the site of the intestinal anastomosis can become stronger and o reduce the risk of intestinal leakage.

CONCLUSION

The use of free peritoneal patch on colon anastomosis in intraperitoneal infection can increase fibroblasts and collagen levels compared to those without the use of free peritoneal patch.

CONFLICTS OF INTEREST

No competing interests declared.

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ETHICAL STATEMENT

This study has been approved by Ethical Committee Faculty of Medicine Universitas Airlangga/Soetomo Hospital Surabaya, Indonesia.

AUTHOR CONTRIBUTION

All author had contributed to manuscript writing and agreed to for final version of the manuscript for publication.

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