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The effect of bioabsorbable (fibrillar) and fibrin glue on the number of macrophages and fibroblasts in Wistar rats with gastric perforation compared to the omental patch method



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ABSTRACT

Background: Gastric perforation covers 25-30% of acute abdomen in the ER and has high mortality and morbidity rate. Surgery is the primary treatment of gastric perforation. The omental patch is commonly used to close the gastric perforation. One method is to use bioabsorbable material glued with fibrin glue. This study aims to determine the effect of bioabsorbable material as a sutureless technique in wound healing of gastric perforation.

Method: A true laboratory experimental post-test only control group design using a single-blind technique was conducted among 20 Wistar rats. They were divided into 2 random groups, 10 fibrillar and fibrin glue group and 10 omental patch group. Perforation was made by forming a small hole with a diameter of 0.5 cm using a punch biopsy. The microscopic examination with Hematoxylin-eosin staining was carried out for the number of macrophages and fibroblasts from the gastric perforation site. Data were analyzed using SPSS version 16 for Windows.

Result: The average number of fibroblasts produced by the fibrillar and fibrin glue group was 7.5 and 5.4 in omental patch group. Based on the result of T-test (fibroblasts), it was obtained that there was a significant difference (H1 is accepted) in the number of fibroblasts produced using fibrillar and fibrin glue compared to the omental patch method ($P < 0.001$). The average number of macrophages in fibrillar and fibrin glue group was 4 and 3 in omental patch group. Based on T-test, it was found a significant difference in the number of macrophages produced using fibrillar and fibrin glue compared to the omental patch method ($P < 0.05$).

Conclusion: The administration of fibrillar and fibrin glue biomaterials in the closure of gastric perforation increased the number of macrophages and fibroblast cells better than the omental patch method.

Keywords: Bioabsorbable, fibrin glue, macrophages, fibroblast, omental patch

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INTRODUCTION

Gaster is a gastrointestinal organ with essential digestive function, nutrition and endocrine.¹ This organ stores and facilitates the digestion and absorption of food and helps appetite regulation. The gastric disease is a common pathological condition, and gaster is an organ that relatively responds well to therapy.¹

One of the most common gastric conditions that often occurs is gastric perforation.² Gastric perforation covers 25-30% of acute abdominal diseases in the ER and has a high mortality and morbidity rate.² A previous data states that deaths from gastric perforation in the world during 14-year of study reached 5.8% at hospital postoperatively.³ Gastric perforation is defined as the formation of a hole in the wall of part of the gastrointestinal tract, where the hole allows gastric contents to enter abdominal cavity and cause peritonitis. The most common aetiology of gastric perforation is ulcers, with an

incidence of perforation between 2-10% of all patients with peptic ulcer.⁴ Other causes of gastric perforation are trauma, neoplasms, ingestion of foreign and iatrogenic objects.⁴

Currently, the incidence of gastric perforation is increasing in the elderly population (60-70 years). It is often associated with NSAID use in the elderly population.⁵ Perforation develops into chemical peritonitis due to gastric acid leakage into the abdominal cavity, and within a few hours, bacterial contamination occurs. Gastric perforation is a life-threatening condition, so that early diagnosis and treatment shall be immediately performed.^{4,5}

The treatment for gastric perforation cases is primarily surgery.⁶ But the real truth on the ground, not all cases of gastric perforation can be immediately performed by definitive surgery, for example in septic shock condition. Definitive procedures are delayed until the patient's condition

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improves. While performing supportive therapy, intraperitoneal drainage is installed as the source of control. Another consideration for the installation of intraperitoneal drainage is the increased incidence of gastric perforation in high-risk groups.⁶ The population included in the high-risk group are those who are at the age of > 60 years and have other comorbidities. Postoperative mortality rates in high-risk patients reach >50%.⁶ Patients classified with Boey score >2 are classified as patients with poor prognostic value, so that it is necessary to consider minimum operative management, such as the installation of intraperitoneal drainage as source control and providing an opportunity for the tissues to heal.

The omental patch is commonly used for the closure of gastric perforation.⁷ Exploration laparotomy, and omental patch remains the gold standard until this day. Omentum has numerous macrophages which play a role in phagocytosis of bacteria and dead tissue.⁷ In addition, macrophages have the ability to secrete various growth factors. These growth factors play a role in the migration and proliferation of fibroblasts. The disadvantage in this technique is that sutures on the edge of perforation are fragile, which is prone to leakage.⁷

Laparoscopy in the treatment of gastric perforation was first introduced in 1990 and has many advantages over open laparotomy, so that this new technique is relatively popular.⁸ The disadvantages of this procedure are longer operating time and requiring considerable time due to the difficulty of suturing per laparoscopy.⁸

As an alternative to omental patch, the method to close the perforation without injuring the edges of perforation has been considered, so that it does not pose a risk of leakage. One approach is to use bioabsorbable material glued with fiber glue. Implantation of bioabsorbable material (fibrillar) in the body stimulates a body's reaction to a foreign object, and the biomaterial is coated by a protein called opsonin.⁷ Opsonin then triggers the induction of cytokines that attract macrophages around the biomaterial. Macrophages secrete chemical mediators that stimulate the formation of fibroblasts.⁷

Fibroblasts are cells that play an important role in wound closure.⁹ Fibroblasts begin to migrate on the third day and reach its peak in the second week after the perforated wound. The primary function of fibroblasts is to create collagen as the primary component of the extracellular matrix. The extracellular matrix will be formed into fibrotic tissue to close the wound.⁹

The main problem in the treatment of gastric perforation is finding an efficient and safe therapeutic approach. Based on those mentioned above,

this study was conducted to determine the effect of bioabsorbable material as a sutureless technique in wound healing of gastric perforation.

METHOD

A true laboratory experimental post-test only control group design using single-blind was conducted among 20 Wistar rats. This study was carried out at the Pharmacology Laboratory and Anatomy Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

Samples were taken randomly from male Wistar rat population as experimental animals with an average weight of 200-300 grams, aged 3-4 months. The samples consisted of 2 groups, namely rat with gastric perforation treated with fibrillar and fibrin glue group and rat with gastric perforation treated with omental patch group. About 10 rats were evaluated for each treatment group, plus 1 rat as a reserve. Therefore, the number of animals needed for 2 treatments was 20 rats.

The experimental animals were randomly divided into 2 groups, namely fibrillar and fibrin glue group and omental patch group. In both groups, laparotomy was performed and perforation was made on the stomach. Perforation was made by forming a small hole with a diameter of 0.5 cm using a punch biopsy. In the first group, the closure of perforation was carried out using fibrillar material glued with fibrin glue. In the second group, the closure of perforation was carried out with omental patch. In the second week, specimens were collected from each group for histopathological examination. In this study, microscopic examination with Hematoxylin eosin staining for the number of macrophages and fibroblasts from the gastric perforation site that began healing with a numerical scale was performed and analyzed. In order to compare the number of macrophages and fibroblasts from each group, the Independent Samples T-test was performed using the SPSS software version 16 for Windows. A p-value was determined statistically significant if less than 0.05.

RESULT

In [Table 1](#), it can be seen that the average number of fibroblasts produced using fibrillar and fibrin glue was 7.50 ± 0.52 fibroblast cells per field of view, while the average number of fibroblasts produced using the omental patch method was 5.40 ± 0.69 fibroblast cells per field of view. This result indicated that the average number of fibroblasts produced using fibrillar and fibrin glue method was higher than those produced using the omental patch method. Due to data normally distributed (Kolmogorov-Smirnov

Table 1 The comparison of fibroblast and macrophages cells between the treatment groups

Variables	Treatment Group (N=20) (Mean±SD)		Normality Test	Levene's Test	P
	Fibrillar-Fibrin Glue (N=10)	Omental Patch (N=10)			
Fibroblast (cells/field)	7.50±0.52	5.40±0.69	0.595	0.303	0.000
Macrophages (cells/field)	4.00±0.67	3.00±0.67	0.253	1.000	0.004

non-parametric test) and homogeneous (Levene's test) ($P>0.05$), independent T-test was used to evaluate the difference between both groups. Our study found a significant difference in the number of fibroblasts produced using Fibrillar and Fibrin glue, and those produced using the Omental patch method ($P=0.000$) (Table 1)

T-test for two groups of independent samples was conducted to test whether there is a significant difference between the average number of macrophage cells between fibrillar and fibrin glue group and omental patch group. From the Table 1, it can be seen that the average number of macrophage cells in fibrillar and fibrin glue group is 4.00 ± 0.67 macrophage cells per field of view, and the average number of macrophage cells in omental patch group is 3.00 ± 0.67 macrophage cells per field of view. Thus, it can be seen that the average number of macrophage cells in fibrillar and fibrin glue group is higher than in omental patch group. Besides, based on the Independent T-test, there was a significant difference in the number of macrophage cells produced using fibrillar and fibrin glue on the omental patch method ($P=0.004$) (Table 1).

DISCUSSION

The calculation of the number of fibroblasts and macrophage in histopathological preparations was carried out in both groups using 400x magnification, then the difference in the number of fibroblasts and macrophage was compared between closure of gastric perforation using fibrillar and fibrin glue group and closure of gastric perforation using omental patch group, then it was performed statistical analysis using T-test to compare the number of fibroblasts and macrophages produced by both groups. This study indicated that the average number of fibroblasts and macrophages produced by fibrillar and fibrin glue group is higher than the average number produced by omental patch group.

Macrophages are cells that participate in phagocytosis of bacteria and dead tissues such as PMN, but macrophages have another vital function in wound healing, namely the recruitment of other cells through cytokines and growth factor.¹⁰ Macrophages release mediators such as TGF- β , VEGF, PDGF, EGF, and FGF which function in the

process of cell proliferation, matrix synthesis and angiogenesis.^{10,11} These mediators play a significant role in fibroblast formation.

Therefore, to understand the role of fibrillar and fibrin glue as well as the omental patch in wound closure of gastric perforation, it is necessary to calculate macrophage cells between the two treatment groups.

From the results of the calculation of macrophage cells, it was obtained that the average number of macrophages was higher in fibrillar and fibrin glue group compared to the omental patch group. This is in accordance with the mechanism of action of bioabsorbable material of fibrillar, namely when a bioabsorbable material is in contact with plasma or blood, the bioabsorbable material is coated by a protein called opsonin.⁷ Opsonin then triggers proinflammatory cytokines and releases inflammatory cells.⁷

If the inflammatory process continues and bioabsorbable material remains in the implant site, the body reacts to a foreign object, at this point, the macrophages combine to form multinucleated cells surrounding the fibrillar implant site.¹² Macrophages secrete chemical mediators stimulating the formation of cells that play an important role in wound healing.^{10,11} One of the mediators secreted by macrophages is PDGF (Platelet-derived growth factor), PDGF is a chemotactic factor for fibroblasts.¹³ The proliferation phase of wound healing roughly begins on the fourth day until it reaches its peak in the fourteenth day.¹⁴

However, in the omental patch method, where the omentum has an abundant supply of angiogenesis material, human omental microvascular endothelial cells (HOME cells) can contain the same peptide structure as FGF (fibroblast growth factor).¹⁵ FGF stimulates the formation of fibroblasts which play a role in the wound healing granulation process.^{16,17}

Because fibrillar is a bioabsorbable material defined as a natural or synthetic material made to interact with a biological system or living tissue for medical purposes, the closure of gastric perforation using bioabsorbable materials of fibrillar and fibrin glue is highly possible to apply to humans. In addition, the closure of gastric perforation using fibrillar and fibrin glue method does not cause leakage, has faster operation time and facilitates laparoscopy.¹⁸

From previous study with different material from this study, namely the effect of administration

of Lactic Glicolide Caprolactone (LGC) on the increase in number of fibroblasts in the wound healing process of gastric perforation, the same result was obtained where there was an increase in the number of fibroblasts in the administration of LGC compared to the omental patch method.

CONCLUSION

The administration of fibrillar and fibrin glue biomaterials in the closure of gastric perforation increased the number of macrophage cells and fibroblasts better than the Omental Patch method. The closure of gastric perforation using fibrillar and fibrin glue biomaterials has the potential to be an alternative therapy for gastric perforation.

CONFLICT OF INTEREST

The authors declared that there was no conflict of interest in this study.

ETHICAL CLEARANCE

This research had been approved by the Ethics committee prior to the study conducted.

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AUTHOR CONTRIBUTION

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

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