



# Comparison of classical surgery and sutureless repair with DuraSeal or fibrin glue for duodenal perforation in rats

Siçanlarda oluşturulan duodenum perforasyonunda klasik cerrahi ile DuraSeal ya da fibrin yapıştırıcı ile yapılan dikişsiz onarımların karşılaştırılması

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## BACKGROUND

The purpose of the study was to compare classical primary suture repair and sutureless repair with fibrin glue or DuraSeal adhesion barrier for the closure of duodenal perforation in rats.

## METHODS

Forty adult female Wistar Albino rats weighing between 250-300g were randomly divided into four equal groups. Primary repair, primary repair and omentoplasty, or application of fibrin glue or DuraSeal adhesion barrier was performed in each of the four groups, respectively. The bursting pressure, tissue hydroxyproline levels and histopathology were evaluated.

## RESULTS

Bursting pressure values of the primary repair and primary repair and omentoplasty groups were significantly higher than in the fibrin glue and DuraSeal groups ( $p<0.001$ ). There were no significant differences between the experimental groups regarding hydroxyproline levels and histological parameters.

## CONCLUSION

The sutureless methods (Fibrin glue, DuraSeal) have no superior effects when compared with the conventional repair techniques. We observed similar results between the sutureless repair groups; thus, DuraSeal can be considered an alternative to fibrin glue for this purpose. This suggestion must be supported with new studies, however, which would be planned with other wound healing markers and different designs.

**Key Words:** DuraSeal; duodenal perforation; fibrin glue; sutureless repair.

## AMAÇ

Bu çalışmada, siçanlarda oluşturulan deneysel duodenum perforasyonunda klasik cerrahi teknikler ile adezyon bariyerleri olan DuraSeal ya da fibrin yapıştırıcıyla yapılan dikişsiz onarım tekniklerinin karşılaştırılması amaçlandı.

## GEREÇ VE YÖNTEM

Kırk adet yetişkin, ağırlıkları 250-300 g arasında olan, dişi Wistar Albino siçan rastgele dört adet eşit gruba ayrıldı. Primer onarım grubu, primer onarım ve omentoplasti grubu, fibrin yapıştırıcı uygulanan grup ve DuraSeal uygulanan grup. Doku iyileşmesini değerlendirmek amacıyla patlama basıncı, doku hidroksiprolin seviyesi ve histopatolojik inceleme parametre olarak kullanıldı.

## BULGULAR

Primer onarım, primer onarım ve omentoplasti gruplarının patlama basıncı değerleri fibrin yapıştırıcı ve DuraSeal grubu patlama basıncı değerlerine göre anlamlı olarak yüksekti ( $p<0,001$ ). Biyokimyasal ve histolojik parametreler açısından tüm gruplar arasında anlamlı farklılık yoktu.

## SONUÇ

Değerlendirdiğimiz dikişsiz onarım tekniklerinin konvansiyonel onarım tekniklerine üstün etkilerinin olmadığını gözlemledik. Dikişsiz onarım gruplarının sonuçları benzerdi. DuraSeal, fibrin yapıştırıcı gibi dikişsiz onarım alanında alternatif olabilir. Bu çıkarım farklı yara iyileşmesi belirteçleri ve farklı yöntemlerle planlanmış çalışmalarla desteklenmelidir.

**Anahtar Sözcükler:** DuraSeal; duodenal perforasyon; fibrin yapıştırıcı; dikişsiz tamir.

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Duodenal perforation is an urgent situation, which usually necessitates surgical treatment.<sup>[1]</sup> Peptic ulcer disease is the most common cause of duodenal perforation.<sup>[1,2]</sup> Peptic ulcer disease is a frequent pathology that is caused by different disorders. *Helicobacter pylori* infection, nonsteroidal anti-inflammatory drugs, liver cirrhosis, hyperparathyroidism, and chronic pancreatitis are related to peptic ulcer disease.<sup>[3,4]</sup> Definitive surgical approaches for the management of peptic ulcer became archaic after the use of proton pump inhibitors (PPIs) and expansion of *H. pylori* eradication therapy. In suitable conditions, primary repair is the preferable technique for duodenal perforations.<sup>[5]</sup> Mortality and morbidity rates increase with delayed or failed surgery. Primary repair can be performed with classical open surgical technique or laparoscopy for duodenal peptic ulcer perforations. As a minimally invasive approach, sutureless repair of duodenal peptic ulcer perforation can simplify the technique and reduce the operation time.

Fibrin glue is used in clinical practice for duodenal perforations as an alternative sutureless repair via laparoscopic or endoscopic route.<sup>[6,7]</sup> Fibrin glue is obtained from human fibrinogen concentrate and used to support wound healing.<sup>[8]</sup> Fibrin glue mimics the last episode of coagulation. Thrombin and aprotinin are the main components of fibrin glue. Thrombin converts fibrinogen to fibrin. Factor XIII binds fibrin monomers with covalent links for clotting. Aprotinin is added to fibrin glue to prevent fibrinolysis.<sup>[8,9]</sup> It has been reported that fibrin glue prevents hematoma formation and supports fibroblast activity and angiogenesis.<sup>[10,11]</sup> Fibrin glue is being used in various pathologies including upper gastrointestinal tract perforations,<sup>[7,12]</sup> fistulas<sup>[13,14]</sup> and leaks.<sup>[15,16]</sup>

DuraSeal, which is an absorbable synthetic hydrogel, has blue solution and limpid solution. DuraSeal is a newly introduced material aiming to minimize leaks in various organs, such as cerebrospinal fluid in neurosurgery, air in thoracic surgery and blood in vascular surgery.<sup>[17-21]</sup>

The present experimental study aimed to compare the primary closure of duodenal defects by various techniques, notably primary repair, primary repair and omentoplasty, and DuraSeal or fibrin glue application.

## MATERIALS AND METHODS

This study was performed after approval of the Ethics Committee of the Animal Care Review Board of Istanbul University, Experimental Medicine Research Institute. Adult female Wistar Albino rats, weighing 250-300 g, were obtained from Istanbul University Cerrahpasa Medical Faculty Experimental Animals Research Laboratory. The rats were cared for in accordance with the Guide for the Care and Use of Labo-

ratory Animals (NIH Publication no. 86-23, revised 1985), maintained in colony cages (5 or 6 per cage) under controlled conditions of temperature (28°C), light (10 h light: 14 h dark) and humidity (50°F 5%). The rats were not permitted ad libitum access to standard lab chow and tap water starting from 12 hours before the surgery to the end of the experimental procedures. 10% dextrose solution (10 ml/day per rat) was given to the rats intraperitoneally during the postoperative period. Under ketamine chloride (40 mg/kg) anesthesia, the abdominal area was cleansed with povidone iodine solution after shaving. A median abdominal incision was performed. The anterior wall of the first part of the duodenum was perforated (0.2 cm) with a scalpel in every rat. The abdominal incision was closed with 3/0 silk continuous sutures as a single layer. Forty rats were divided randomly into four weight-matched equal groups as follows: primary repair (with 4/0 silk) group (n=10), primary repair and omentoplasty group (n=10), fibrin glue group (n=10) (Beriplast P combi set, ZLB Behring AG, Marburg, Germany; glue was sprayed on the perforated area without suturing), and DuraSeal group (n=10) (Confluent Surgical, Inc., Waltham, MA; DuraSeal was sprayed on the perforated area without suturing). The components of fibrin glue and DuraSeal were carefully reconstituted and prepared according to the manufacturers' instructions. Sufficient (0.25 ml) equal volume of fibrin glue and DuraSeal to entirely cover the perforated area was applied to a target surface. For covering the perforated area surfaces, the fibrin glue and DuraSeal were sprayed using the enclosed spray-tips. The rats were sacrificed on postoperative day 4 with overdose ether inhalation. After the measurement of bursting pressure levels of the repaired duodenal perforation site, an en-bloc excision of the repaired area together with 0.5 cm proximal and distal parts of the duodenum was performed. The excised part of the duodenum was divided into two equal tissue samples for histopathological and tissue hydroxyproline level analysis. For tissue hydroxyproline level investigation, samples were immediately immersed in liquid nitrogen and stored at -70°C until being processed, and for histopathological evaluation, samples were fixed in 10% formaldehyde solution.

### Measurement of Bursting Pressure

The abdominal incision of the rats was opened and the adhesions around the repaired area were preserved. The small intestine was ligated 2 cm distal from the repaired area of the duodenum. A catheter was inserted from the distal esophagus and fixed with 2/0 silk suture. The prepared system was sunk into a bowl filled with water. Air was insufflated with 6 ml/min stable speed and the bursting pressure was measured with a sphygmomanometer. The pressure level when bubbles were first seen in the water was accepted as the bursting pressure level.

### Hydroxyproline Assay

The samples for hydroxyproline levels were weighed, cut into small pieces, and homogenized in a phosphate buffer to yield a 20% homogenate. Aliquots of the homogenate were added to an equal volume of 6 N hydrochloric acid, and hydrolyzed in Teflon-capped vials at 102°C for 16 hours. The hydroxyproline content of the tissue hydroxylates was determined spectrophotometrically by using the standard addition method developed by Kivirikko et al.<sup>[22]</sup> (Hypopronosticon Kit lot/ch. B:E 92401; Organon Teknika B.V., Boxtel, Holland). Results were expressed in milligrams, such as in hydroxyproline/100 mg (wet weight).

### Histopathological Evaluation

Duodenal tissue slices were then fixed in 10% buffered formalin and embedded in paraffin. Each section in 4 µm thickness was stained with hematoxylin and eosin for light microscopic assessment. A certified pathologist scored samples in a blinded fashion. An arbitrary scope was given to each microscopic field at magnifications of 20x, 40x and 100x. Ten representative areas from each section consisting of inflammation, revascularization, fibroblasts and collagen were examined, and scored to obtain the mean value. The parameters were graded on a 3-grade scale; mild, moderate or marked changes were indicated by an increase or decrease of 1, 2 or 3 grades, respectively.

### Statistical Evaluation

All the values were expressed as the mean ± SD. The data of bursting pressure and hydroxyproline content were analyzed by ANOVA test followed by a multiple comparison post-hoc test of Tukey. Mann-Whitney U test was used for the evaluation of the histopathological data. Values were considered as significant when p<0.05. SPSS 12 (SPSS Inc. Chicago, IL, USA) was used for statistical analyses.

## RESULTS

Six rats in total died: 3 each in the fibrin glue and the DuraSeal groups. One in each of these groups died immediately after induction of anesthesia. The other 4 rats died on postoperative day 2. Macroscopic leakage was observed in 2 rats of the fibrin glue group. However, no peritonitis and no leakage were observed in the other 2 rats in the DuraSeal group. Additional rats were included into the study to replace the rats that died. Intraabdominal adhesions were seen in all experimental groups. Bursting pressure levels are summarized in Table 1. There were no significant differences between the conventional repair groups (primary repair group, primary repair and omentoplasty group) with regard to bursting pressure levels. Bursting pressure levels of the conventional repair groups were significantly higher than in the sutureless repair groups (fibrin glue group and DuraSeal group)

**Table 1.** Bursting pressure levels according to treatment groups

Experimental groups	Bursting pressure (mm/Hg)
Primary repair (n=10)	166±15.78
Primary repair+omentoplasty (n=10)	185±42.49
Fibrin glue (n=10)	84.29±11.34 ***###
DuraSeal (n=10)	91.43±6.90 ***###

\* Significance between the primary repair group and the other groups defined with (\*), (p<0.001=\*\*\*).  
 # Significance between the primary repair+omentoplasty group and the other groups defined with (#), (p<0.001=###).

**Table 2.** Tissue hydroxyproline levels according to the treatment groups

Experimental groups	Tissue hydroxyproline levels (µg/g wet tissue)
Primary repair (n=10)	960.79±697.67
Primary repair+omentoplasty (n=10)	490.45±224.97
Fibrin glue (n=10)	599.12±447.35
DuraSeal (n=10)	732.73±740.11

(p<0.001). There were no significant differences between the fibrin glue and DuraSeal groups regarding bursting pressure levels.

Hydroxyproline levels of the experimental groups are summarized in Table 2. There were no significant differences between groups regarding tissue hydroxyproline levels.

Revascularization, inflammation and number of fibroblasts were similar between the primary repair and primary repair and omentoplasty groups. The collagen amount in the primary repair group was higher than in the other groups. Neoangiogenesis and number of fibroblasts were lower in the fibrin glue group. Irregular collagen arrangement was observed rarely in the DuraSeal group; however, there were no significant differences between groups with respect to histopathological evaluation (Table 3).

## DISCUSSION

Surgical treatments of duodenal perforations vary from primary repair to sutureless minimally invasive approaches.<sup>[2,7,23]</sup> The technical difficulties of suturing in laparoscopic and endoscopic approaches have led to research studies about sutureless repair for duodenal perforations.<sup>[7,23,24]</sup>

Application of effective sutureless repairing techniques for intestinal perforations is being studied with new chemical agents to create a proper minimally invasive technique. The effective chemicals, which have adhesive barrier functions, would add to the newest therapeutic approaches in the field of minimally invasive surgery by simplifying the procedures. However,

**Table 3.** Scores of the histopathological evaluation according to treatment groups

Groups	Inflammation	Revascularization	Fibroblast count	Collagen value
Primary repair (n=10)	1.5±0.53	2.1±0.55	2.5±0.53	2.5±0.53
Primary repair + omentoplasty (n=10)	1.6±0.7	2.1±0.74	2.2±0.63	2.3±0.43
Fibrin glue (n=10)	1.5±0.53	2±0.67	2.5±0.53	2.5±0.53
DuraSeal (n=10)	1.6±0.7	2±0.82	2.33±0.57	2.3±0.89

this hypothesis should be evaluated in experimental studies initially.

The first laparoscopic repair technique was introduced with primary repair and omentoplasty in 1989.<sup>[25]</sup> After this approach, many different methods were performed, such as omental patch with fibrin plug,<sup>[26]</sup> repair with ligamentum teres hepatis,<sup>[27]</sup> fibrin glue,<sup>[7]</sup> omental patch application with stapler,<sup>[28,29]</sup> and fibrin patch (TachoComb).<sup>[30]</sup> The beneficial effects of fibrin on wound healing have been shown.<sup>[31]</sup> It has been reported that laparoscopic fibrin glue application for duodenal ulcer perforation has similar postoperative results to primary repair or primary repair with omentoplasty. Additionally, fibrin glue application minimized the operating time.<sup>[6,7,24]</sup> Easy application and effective barrier function are the main components that reduce operating time. Lau et al.<sup>[7]</sup> suggested that sutureless repair of duodenal perforations is as safe as conventional techniques and is easy to learn for the treatment of the duodenal perforations that are smaller than 1 cm.

Prior to the present study, no data were available about the effects of DuraSeal application for gastrointestinal perforations. We observed similar results regarding histopathological and hydroxyproline levels in all the experimental groups. When we evaluated the bursting pressure levels, conventional repair methods were safer and more secure than the sutureless repair techniques. The sutureless methods that we evaluated were shown to have no superior effects to conventional repair techniques. As a foreseeable result, suturing caused physical stability in the repaired area. The serosal surface, which is the most potent layer of the intestine, is closed with suturing. In sutureless repair, the defect is clogged with a chemical agent. Especially in the early phase of the wound healing process, the physical stability could be weaker in the repaired area. In clinical practice, physical straight is not a sine qua non parameter of the repair of duodenal perforation. In the literature, some methods, such as Taylor's method, which require no suture application or surgical intervention, have been described for the treatment of duodenal perforations. In Taylor's method, nasogastric aspiration, fluid resuscitation, parenteral broad-spectrum antibiotics, and antisecretory drugs are used. Taylor's method is suggested as an alternative in selected cases of perforated gastroduodenal ulcers, the main advan-

tage being the avoidance of anesthetic and surgical stress, with their potential morbidity and mortality.<sup>[32]</sup> However, using an adhesive barrier that can repair perforations or leaks quickly and easily would be more logical for the surgeons than leaving the perforation untouched.

Fibrin glue is being used in clinical practice in the sutureless treatment of duodenal perforations.<sup>[7]</sup> All the procedures evaluated in this study have been used in clinical practice, except DuraSeal. Hydroxyproline levels and histological evaluation were similar in all the experimental groups. This result reminds us that there was no difference between groups during the wound healing process. According to this result, we suggest that DuraSeal can be considered an alternative material for the sutureless treatment of duodenal perforation, like fibrin glue, because we did not observe any harmful or worse effect of DuraSeal when compared with fibrin glue. However, this hypothesis must be supported by new studies that are planned with other wound healing markers and different designs.

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