*Original Article*

Archives of Clinical & Experimental Surgery

# Preventing Clinical Leakage of Colonic Anastomoses with A Fibrin - Coated Collagen Patch Sealing - An Experimental Study

*Tyge Nordentoft, Kathrine Holte*

Department of
Gastrointestinal Surgery
Section 360
Hvidovre University Hospital
Denmark

Received: December 07, 2012
Accepted: March 21, 2013
Arch Clin Exp Surg 2014;3:201-206
DOI:10.5455/aces.20130321071651

Corresponding author:
Tyge Nordentoft, MD
Consultant Surgeon
Department of
Surgical Gastroenterology
Section 360
Hvidovre University Hospital
DK-2650 Hvidovre, Denmark
nordentoft@dadlnet.dk

**Abstract**

**Background:** Anastomotic leakage remains a major complication following colorectal surgery, with a largely unknown pathophysiology and limited treatment options. Previous clinical studies have revealed that many leakages on colo-rectal anastomoses remain subclinical. Prior attempts at prophylactic mechanical sealing of gastrointestinal anastomoses, i.e., the application of various forms of mesh and fibrin components, have been disappointing. We therefore decided to determine whether a collagen patch coated with fibrin glue components (TachoSil®) is able to seal leaking colonic anastomoses and thereby prevent clinical leakage and peritonitis.

**Material and methods:** Prospective study on 20 pigs operated with experimentally induced defects in colonic anastomoses randomized to sealing vs. no sealing with a collagen patch coated with fibrin glue components. The primary study endpoints were visible leakage at the anastomotic site, death or illness causing sacrifice and fecal peritonitis (local or diffuse).

**Results:** A significant reduction in macroscopic anastomotic leakage in the group of pigs with sealed anastomoses was found (2/10 vs. 9/10, p=0.0055). Furthermore, macroscopic examination of the abdominal cavity (n=20) showed a significant decrease in peritonitis in the sealed vs. non-sealed groups (2 vs. 9, p=0.0055). Additionally, a reduction, although non-significant, in dead and sacrificed animals before the end of the observation period (1 vs. 4) was found in the sealed group.

**Conclusion:** A collagen patch coated with fibrin glue components efficiently seals leaking gastrointestinal anastomoses in pigs. Whether these results may be applied to humans in order to prevent clinical anastomotic dehiscence must be investigated in future randomized clinical studies.

***Key words:*** *Anastomosis, sealing, fibrin glue, leakage, collagen patch*

## Introduction

Anastomotic leakage remains a major complication following gastrointestinal surgery, with reported incidences up to 19% depending on the surgical procedure and patient comorbidity [1,2], and with severe consequences in terms of poor short- as well as long-term outcome [3-9]. The pathogenesis remains largely unknown, though some demographic data (e.g., rectal surgery and cardiovascular comorbidity) have been shown to correlate with an increased leakage rate [1,10,11]. Perioperative interventions such as bowel preparation, temporary stoma creation or drain insertion do not influence the incidence of anastomotic leakages [1, 12,13]. Treatment options are limited to reversal of the

anastomosis with subsequent stoma formation (in patients with clinical signs of peritonitis), or conservative treatment, depending on the site of leakage and clinical condition of the patient. Previous clinical studies have revealed that many leakages on colo-rectal anastomoses remain subclinical [14-16].

In attempts to reduce the incidence of anastomotic leakage, several studies involving attempted mechanical sealing of gastrointestinal anastomoses with various devices, including omental flaps and meshes, have been performed; the results, however, have been unconvincing [17-42]. A collagen patch coated with fibrin glue components (TachoSil®) has been developed and marketed specifically as a hemostatic agent [43,44]. From the limited success seen with liquid fibrin glue components as well as various meshes to seal anastomoses, the hypothesis emerged that combined sealing with a collagen patch coated with fibrin glue components may benefit anastomotic healing as well as reduce clinical leakage.

Consequently, we conducted an experimental study in order to determine whether a collagen patch coated with fibrin glue components could seal leaking colonic anastomoses, thereby preventing peritonitis.

**Materials and Methods**

Prospective study on 20 LYD (Landrace x Yorkshire x Duroc) pigs, with mean weight being 38.0 kg. Following premedication with midazolam, laparotomy was performed under general anesthesia, maintained with propofol and fentanyl. The colon was divided, resected 2 cm, and an end-to-end, double-layer anastomosis was made with interrupted absorbable polyfilament suture (Vicryl® 3-0). Before completion of the double-layer suture, a standardized defect in the anastomosis was made by inserting a tube of 21 mm in diameter through the anastomosis from the outside. The remaining sutures were then placed around the tube, which was subsequently removed in order to produce a standardized defect. The size of the tube was based on previous experimental findings of a defect made by a tube of 21 mm in diameter, resulting in significant anastomotic leakage and peritonitis [45]. Following formation of the standardized defect, the 20 pigs were randomized into two groups of 10 pigs each to sealing vs. no sealing of the defect. Randomization was achieved by the sealed-envelope method. In the pigs randomized to sealing, the entire anastomotic line was sealed with a 9.5cm x 4.8cm collagen patch coated with fibrin glue components (TachoSil®, Nycomed, Zurich, Switzerland). TachoSil® consists of an equine collagen patch coated with the fibrin glue components — human fibrinogen and human thrombin. TachoSil®, originally marketed as a hemostatic product, is approved for human use by the European Medicines Agency (EMA) and comes in a sterile packing for intraoperative use. In the pigs randomized to no sealing, the anastomoses with the standardized defect were left untouched. Following the sealing vs. no sealing procedure, the abdominal wall was closed in all pigs. Antibiotic prophylaxis took the form of a single preoperative dose of 1ml (25.000 IU)/10kg procaine benzylpenicillin and dihydrostreptomycin of 50 mg/10 kg.

The pigs were continuously monitored (general behavior, food intake, fecal production, and temperature) for 7 days postoperatively. Animal care and observations during the study period were performed by trained animal technicians, and supervised by veterinarians who attended the animals daily as well as in the onset of illness. The pigs were euthanized on completion of the observation period (7 days postoperatively) or before, if they showed any signs of illness. Euthanasia was performed by injection of 1000mg pentobarbital, and macroscopic examination of the abdominal cavity took place post-mortem.

The primary study endpoints were visible leakage at the anastomotic site, death or illness causing sacrifice, and fecal peritonitis (local or diffuse). One week after surgery, it is likely to believe that some degree of macroscopically and/or histopathologic inflammation will be found, whether there is leakage or not. Because of that, we decided to define peritonitis only as fecal peritonitis, i.e., visible feces in the peritoneum. This might be all over the peritoneum, i.e., diffuse, or just in a part of the peritoneum, e.g., around the anastomosis, i.e., localized.

All surgery was performed by one consultant colorectal surgeon (TN) who is licensed to perform experiments on live animals according to the University of Copenhagen and the Danish Ministry of Justice (license no. 2004-35).

All animals were cared for as per the requirements

of The Danish Animal Experiments Inspectorate, who approved the study (Approval code: 20047561-889).

**Statistics**

Data were analyzed by Fisher's exact test. $P < 0.05$ was considered significant.

**Results**

The results are summarized in Table 1. We demonstrated a significant reduction in macroscopic anastomotic leakage in the group of pigs with sealed anastomoses (2/10 vs. 9/10, p= 0.0055).

Five pigs died or were sacrificed before the end of the observation period. These animals had an autopsy and were all presented with diffuse or local peritonitis upon examination. The remaining 15 pigs completed the observation period, and were sacrificed after 7 days. Macroscopic examination of the abdominal cavity in all of the animals (n=20) showed a significant decrease in peritonitis in the sealed vs. non-sealed group (2 vs. 9, p=0.0055). Furthermore, a reduction, although non-significant, in dead and sacrificed animals before the end of the observation period (1 vs. 4, p>0.05) was found in the sealed group.

In all pigs the collagen patch coated with fibrin glue components (TachoSil®) was found in situ around the anastomosis.

**Discussion**

In summary, we found that a fibrin-coated collagen patch may seal an artificially induced leakage at the site of a colonic anastomosis, with subsequent prevention of peritonitis in an experimental model. Furthermore, a reduction (although non-significant) in death and illness causing sacrifice was found in the animals with sealed anastomoses.

A number of mechanical agents for anastomotic sealing have been evaluated previously, however, with unconvincing results, i.e., sealing of anastomoses with omental flaps has had limited success in both experimental [17,23,30,33,37,42] and clinical studies [18,24,34,40], and attempts at sealing using various forms of mesh have also taken place, but with varying results [19,23,25]. Sealing of colon anastomoses with a polypropylene-type mesh in dogs resulted in a decreased incidence of anastomotic leakage, while no difference was observed following sealing with a polytetrafluorethylene-type mesh [23]. Furthermore, application of a polyglycolic mesh resulted in increased frequency of leakage in the intervention group, possibly attributable to reduced peritoneal or omental contact [25]. Similarly, experimental studies on reinforcement of sutured or stapled gastrointestinal anastomoses with liquid fibrin glue have produced varied results, i.e., one study revealed a reduction in anastomotic leakage [29], while others revealed no difference or even a higher rate of leakage following sealing [20,26-28,35,36,38,41].

Clinical studies involving application of liquid fibrin glue also produced inconclusive results [21,22,31,32,39,46]: one study resulted in no reduction in the incidence of anastomotic leakage compared with a control group following gastric bypass surgery [32], and in a similar, though uncontrolled, study involving 738 patients, only patients whose anastomoses were not sealed with fibrin glue in gastric bypass surgery developed anastomotic leakage (2 patients) [39]. Furthermore, a randomized trial found no advantages of sealing with fibrin glue following pancreaticoduodenectomy [31]. Finally, in two randomized trials involving patients having undergone esophageal resection, no benefit was identified following sealing with liquid fibrin glue [21,22]. No controlled, clinical studies in respect of sealing small bowel, colon or rectum anastomosis have been performed to our knowledge.

Thus, the proposed combination of a mesh coated with fibrin glue may seem a logical development. The aim of the present study was to determine whether a collagen patch coated with fibrin glue components (TachoSil®) is capable of sealing leaking gastrointestinal anastomoses, thereby preventing clinical leakage and peritonitis.

**Table 1:** The results are summarized in table.

| | Leakage | No leakage | Peritonitis (y/n) | Dead/sacrificed before 7 days post-op |
|---|---|---|---|---|
| Sealed group (n=10) | 2* | 8* | 2/8* | 1 |
| Non-sealed group (n=10) | 9* | 1* | 9/1* | 4 |

*Macroscopic anastomotic leakage identified post mortem: Peritonitis refers to faecal peritonitis.

Only a small number of experimental and clinical studies on this form of seal have been published previously. We previously demonstrated the safety of the fibrin glue-coated collagen patch sealing in an experimental model in pigs [47]; however, in a study on small-diameter anastomosis in rats, Chemelnik et al. reported a stenosis of the sealed anastomosis and advised against sealing of small-diameter anastomoses [48]. In contrast, another study of suture-free small-bowel anastomosis in pigs, which was sealed with a fibrin glue-coated collagen patch, reported this method to be safe and feasible [49]. Palentis et al. performed a study on complete and incomplete colonic anastomosis on 206 mice, which was randomized to sealing with a fibrin glue-coated collagen patch (TachoSil®) vs. no sealing, and found improved healing and reduced leakage and lethality in the sealing groups [50].

The present study was performed on a pig model of leaking colon anastomoses validated in a previous study, demonstrating that the experimentally induced anastomotic defect would result in peritonitis if the anastomoses were left unsealed [45]. From the present study, we conclude that a collagen patch coated with fibrin glue components appears efficient in sealing incomplete colonic anastomoses in a pig model. These results are similar to the results of Palentis et al., although this study was performed on mice and with a non-validated model of leakage [50].

Thus, the clinical implications of our results require further investigation in randomized clinical studies in order to determine the potential of this method to prevent clinical anastomotic dehiscence.

**Conflicts of Interest**

The study was supported by an unrestricted grant from Nycomed Denmark, who had no influence on study design, execution or interpretation.

**References**

1. Chambers WM, Mortensen NJ. Postoperative leakage and abscess formation after colorectal surgery. Best Pract Res Clin Gastroenterol 2004;18:865-880.
2. Hyman NH. Managing anastomotic leaks from intestinal anastomoses. Surgeon 2009;7:31-35.
3. Marra F, Steffen T, Kalak N, Warschkow R, Tarantino I, Lange J, et al. Anastomotic leakage as a risk factor for the long-term outcome after curative resection of colon cancer. Eur J Surg Oncol 2009;35:1060-1064.
4. Eberhardt JM, Kiran RP, Lavery IC. The impact of anastomotic leak and intra-abdominal abscess on cancer-related outcomes after resection for colorectal cancer: a case control study. Dis Colon Rectum 2009;52:380-386.
5. Ptok H, Marusch F, Meyer F, Schubert D, Gastinger I, Lippert H. Study Group Colon/Rectum Carcinoma (Primary Tumour). Impact of anastomotic leakage on oncological outcome after rectal cancer resection. Br J Surg 2007;94:1548-1554.
6. Law WL, Choi HK, Lee YM, Ho JW, Seto CL. Anastomotic leakage is associated with poor long-term outcome in patients after curative colorectal resection for malignancy. J Gastrointest Surg 2007;11:8-15.
7. Kube R, Mroczkowski P, Granowski D, Benedix F, Sahm M, Schmidt U, et al. Study group Qualitätssicherung Kolon/Rektum-Karzinome (Primärtumor) (Quality assurance in primary colorectal carcinoma). Anastomotic leakage after colon cancer surgery: a predictor of significant morbidity and hospital mortality, and diminished tumour-free survival. Eur J Surg Oncol 2010;36:120-124.
8. Branagan G, Finnis D. Wessex Colorectal Cancer Audit Working Group. Prognosis after anastomotic leakage in colorectal surgery. Dis Colon Rectum 2005;48:1021-1026.
9. Walker KG, Bell SW, Rickard MJ, Mehanna D, Dent OF, Chapuis PH, et al. Anastomotic leakage is predictive of diminished survival after potentially curative resection for colorectal cancer. Ann Surg 2004;240:255-259.
10. Kingham TP, Pachter HL. Colonic anastomotic leak: risk factors, diagnosis, and treatment. J Am Coll Surg 2009;208:269-278.
11. Suding P, Jensen E, Abramson MA, Itani K, Wilson SE. Definitive risk factors for anastomotic leaks in elective open colorectal resection. Arch Surg 2008;143:907-911.
12. Slim K, Vicaut E, Launay-Savary MV, Contant C, Chipponi J. Updated systematic review and meta-analysis of randomized clinical trials on the role of mechanical bowel preparation before colorectal surgery. Ann Surg 2009;249:203-209.
13. Urbach DR, Kennedy ED, Cohen MM. Colon and rectal anastomoses do not require routine drainage: a systematic review and meta-analysis. Ann Surg 1999;229:174-180.
14. Nesbakken A, Nygaard K, Lunde OC, Blücher J, Gjertsen Ø, Dullerud R. Anastomotic leak following meso-

rectal excision for rectal cancer: true incidence and diagnostic challenges. Colorectal Dis 2005;7:576-581.
15. Karanjia ND, Corder AP, Bearn P, Heald RJ. Leakage from stapled low anastomosis after total mesorectal excision for carcinoma of the rectum. Br J Surg 1994;81:1224-1226.
16. Dorricott NJ, Baddeley RM, Keighley MR, Lee J, Oates GD, Alexander-Williams J. Complications of rectal anastomoses with end-to-end anastomosis (EEA) stapling instrument. Clinical and radiological leak rates and some practical hints. Ann R Coll Surg Engl 1982;64:171-174.
17. Adams W, Ctercteko G, Bilous M. Effect of an omental wrap on the healing and vascularity of compromised intestinal anastomoses. Dis Colon Rectum 1992;35:731-738.
18. Agnifili A, Schietroma M, Carloni A, Mattucci S, Caterino G, Lygidakis NJ, et al. The value of omentoplasty in protecting colorectal anastomosis from leakage. A prospective randomized study in 126 patients. Hepatogastroenterology 2004;51:1694-1697.
19. Aysan E, Dincel O, Bektas H, Alkan M. Polypropylene mesh covered colonic anastomosis. Results of a new anastomosis technique. Int J Surg 2008;6:224-229.
20. Byrne DJ, Hardy J, Wood RA, McIntosh R, Hopwood D, Cuschieri A. Adverse influence of fibrin sealant on the healing of high-risk sutured colonic anastomoses. J R Coll Surg Edinb 1992;37:394-398.
21. Fékété F, Gayet B, Panis Y. [Contribution of fibrin glue to the reinforcement of esophageal anastomoses]. [Article in French]. Presse Med 1992;21:157-159.
22. Fernandez Fernandez L, Tejero E, Tieso A. Randomized trial of fibrin glue to seal mechanical oesophagojejunal anastomosis. Br J Surg 1996;83:40-41.
23. Gulati SM, Thusoo TK, Kakar A, Iyenger B, Pandey KK. Comparative study of free omental, peritoneal, Dacron velour, and Marlex mesh reinforcement of large-bowel anastomosis: an experimental study. Dis Colon Rectum 1982;25:517-521.
24. Hao XY, Yang KH, Guo TK, Ma B, Tian JH, Li HL. Omentoplasty in the prevention of anastomotic leakage after colorectal resection: a meta-analysis. Int J Colorectal Dis 2008;23:1159-1165.
25. Henne-Bruns D, Kreischer HP, Schmiegelow P, Kremer B. Reinforcement of colon anastomoses with polyglycolic acid mesh: an experimental study. Eur Surg Res 1990;22:224-230.
26. Hjortrup A, Nordkild P, Christensen T, Sjøntoft E, Kjaergaard J. Rectal anastomosis with application of luminal fibrin adhesive in the rectum of dogs. An experimental study. Dis Colon Rectum 1989;32:422-425.
27. Hulkko OA, Haukipuro KA, Laitinen ST. Fibrin glue protection of primary anastomosis in the obstructed left colon. An experimental study on the rat. Acta Chir Scand 1988;154:49-52.
28. Kanellos I, Mantzoros I, Goulimaris I, Zacharakis E, Zavitsanakis A, Betsis D. Effects of the use of fibrin glue around the colonic anastomosis of the rat. Tech Coloproctol 2003;7:82-84.
29. Kanellos I, Mantzoros I, Demetriades H, Kalfadis S, Kelpis T, Sakkas L, et al. Healing of colon anastomoses covered with fibrin glue after immediate postoperative intraperitoneal administration of 5-fluorouracil. Dis Colon Rectum 2004;47:510-515.
30. Katsikas D, Sechas M, Antypas G, Floudas P, Moshovos K, Gogas J, et al. Beneficial effect of omental wrapping of unsafe intestinal anastomoses. An experimental study in dogs. Int Surg 1977;62:435-437.
31. Lillemoe KD, Cameron JL, Kim MP, Campbell KA, Sauter PK, Coleman JA, et al. Does fibrin glue sealant decrease the rate of pancreatic fistula after pancreaticoduodenectomy? Results of a prospective randomized trial. J Gastrointest Surg 2004;8:766-772.
32. Liu CD, Glantz GJ, Livingston EH. Fibrin glue as a sealant for high-risk anastomosis in surgery for morbid obesity. Obes Surg 2003;13:45-48.
33. McLachlin AD, Olsson LS, Pitt DF. Anterior anastomosis of the rectosigmoid colon: an experimental study. Surgery 1976;80:306-311.
34. Merad F, Hay JM, Fingerhut A, Flamant Y, Molkhou JM, Laborde Y. Omentoplasty in the prevention of anastomotic leakage after colonic or rectal resection: a prospective randomized study in 712 patients. French Associations for Surgical Research. Ann Surg 1998;227:179-186.
35. Moazami N, Oz MC, Bass LS, Treat MR. Reinforcement of colonic anastomoses with a laser and dye-enhanced fibrinogen. Arch Surg 1990;125:1452-1454.
36. Oka H, Harrison RC, Burhenne HJ. Effect of a biologic glue on the leakage rate of experimental rectal anastomoses. Am J Surg 1982;143:561-564.
37. Pierie JP, de Graaf PW, van Dijk M, Renooij W, van Vroonhoven TJ, Obertop H. Improved healing of extraperitoneal intestinal anastomoses in the early phase when surrounded by omentum. Dig Surg 2000;17:487-491.

38. Saclarides TJ, Woodard DO, Bapna M, Economou SG. Fibrin glue improves the healing of irradiated bowel anastomoses. Dis Colon Rectum 1992;35:249-252.
39. Sapala JA, Wood MH, Schuhknecht MP. Anastomotic leak prophylaxis using a vapor-heated fibrin sealant: report on 738 gastric bypass patients. Obes Surg 2004;14:35-42.
40. Tocchi A, Mazzoni G, Lepre L, Costa G, Liotta G, Agostini N, et al. Prospective evaluation of omentoplasty in preventing leakage of colorectal anastomosis. Dis Colon Rectum 2000;43:951-955.
41. van der Ham AC, Kort WJ, Weijma IM, van den Ingh HF, Jeekel H. Healing of ischemic colonic anastomosis: fibrin sealant does not improve wound healing. Dis Colon Rectum 1992;35:884-891.
42. Volpe M, Costi U, Gianferro A, Albano Barilli R, Marcone P, Sbordoni L. [Use of the omental mesh graft in protecting intestinal anastomoses. Experimental research]. [Article in Italian]. Minerva Chir 1991;46:41-44.
43. Siemer S, Lahme S, Altziebler S, Machtens S, Strohmaier W, Wechsel HW, et al. Efficacy and safety of TachoSil as haemostatic treatment versus standard suturing in kidney tumour resection: a randomised prospective study. Eur Urol 2007;52:1156-1163.
44. Rickenbacher A, Breitenstein S, Lesurtel M, Frilling A. Efficacy of TachoSil a fibrin-based haemostat in different fields of surgery--a systematic review. Expert Opin Biol Ther 2009;9:897-907.
45. Nordentoft T, Sørensen M. Leakage of colon anastomoses: development of an experimental model in pigs. Eur Surg Res 2007;39:14-16.
46. Lim M, Akhtar S, Sasapu K, Harris K, Burke D, Sagar P, et al. Clinical and subclinical leaks after low colorectal anastomosis: a clinical and radiologic study. Dis Colon Rectum 2006;49:1611-1619.
47. Nordentoft T, Rømer J, Sørensen M. Sealing of gastrointestinal anastomoses with a fibrin glue-coated collagen patch: a safety study. J Invest Surg 2007;20:363-369.
48. Chmelnik M, Lasch L, Weih S, Wink E, Romero P, Holland-Cunz S. Anastomotic sealing with a fibrin-coated collagen patch in small-diameter bowel. Langenbecks Arch Surg 2011;396:685-691.
49. Stumpf M, Junge K, Rosch R, Krones C, Klinge U, Schumpelick V. Suture-free small bowel anastomoses using collagen fleece covered with fibrin glue in pigs. J Invest Surg 2009;22:138-147.
50. Pantelis D, Beissel A, Kahl P, Wehner S, Vilz TO, Kalff JC. The effect of sealing with a fixed combination of collagen matrix-bound coagulation factors on the healing of colonic anastomoses in experimental high-risk mice models. Langenbecks Arch Surg 2010;395:1039-1048.