#### **ORIGINAL ARTICLE**



# Comparison of the healing status in suture-less end-to-end intestinal anastomosis using TachoSil with classic two-layer anastomosis in rats

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#### Abstract

The classical two-layer anastomosis has been experimentally compared with anastomosis using synthetic TachoSil in terms of pre- and post-surgical symptoms and healing status of the anastomotic area in rats. Thirty-six adult male rats were randomly allocated into two groups: in group C (control), classic anastomosis was performed in the ileum of the small intestine, 10 cm away from the ileocecal valve using Vicryl 5-0 interrupted suture; group S (study) received anastomosis in the same area but with TachoSil using three sutures (mesenteric, anti-mesenteric, and anterior wall as suture-less method). The obtained results were analyzed by SPSS20 software, using Chi-square test. Based on the obtained results, group C had more favorable anastomotic healing than group S. Actually, the fibroblast and collagen were seen in most cases of the control rats, while healing was stopped in group S (TachoSil group) in early stages due to thrombosis and vasculitis gangrene. According to the findings, TachoSil patch cannot be advised for safe use in suturing the less small-bowel anastomosis.

Keywords Anastomosis · Experimental trial · TachoSil · Rat · Peristalsis · Anastomotic leakage · Dilation

# Introduction

Intestinal anastomosis is one of the most commonly performed surgical procedures and its techniques have always

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Iman Shamohammadi imanshamohammadi@yahoo.com attracted great interest (Bhanot and Alex 2002). Intestinal surgery is the main part of many surgical procedures on the digestive system; also, a reliable, safe, and fast anastomosis is of great importance, especially in the case of hemodynamically

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instability (Buchanan et al. 2003), peritonitis due to perforation, or inadequate blood supply to the anastomosis site caused by vascular disease (Jackson 2001). Moreover, the side effects of the absorbable and non-absorbable sutures at the anastomosis site cannot be ignored (Lau et al. 1996). All these make a reliable and safe anastomosis important (Lau et al. 1996). Short duration of surgery and anesthesia can affect postoperative morbidity and mortality rates in patients with hemodynamic instability (Stumpf et al. 2009). The surgical site leakage is one of the most important issues commonly investigated after surgical operations and can result in adverse effects in the case of negligence (Lau et al. 1996).

The standard technique is the hand-sewn single-layer interrupted suture in small size intestinal bowel. Critically ill neonates benefit from shorter operating time. Various experimental models were developed, such as sealing as well as glueing intestinal anastomoses with fibrin-coated collagen patches such as TachoSil<sup>TM</sup> (Stumpf et al. 2009; Nordentoft et al. 2007).

The safety of sealing small bowel anastomoses with TachoSil<sup>™</sup> and the technical feasibility of suture-free, TachoSil<sup>™</sup>-glued small bowel anastomoses (Stumpf et al. 2009) has been experimentally demonstrated.

Therefore, various anastomotic methods have been employed to control the leakage and shorten the duration of surgery. Herein, two anastomotic methods including classical and TachoSil anastomoses have been compared.

#### Materials and methods

In the present experimental trial, 36 Wistar-albino male rats weighing 250–300 g with the same age, sex, race, and weight were purchased from Kerman University of Medical Sciences animal laboratory and allocated into two groups including group C (control, n = 18) and group S (study, n = 18). Their health status screening was performed under standard laboratory condition: temperature 20–24 °C and 12/12-h light/dark cycle. Each rat was housed in separate cages and fed with pellet and appropriate water at libitum. There was no preoperative or postoperative fasting period. The study was approved by Kerman University of Medical Sciences local ethics committee.

Before laparotomy, the rats were anesthetized with intramuscular injection of ketamine (50 mg/kg; Alfasan International, Woerden, the Netherlands) and xylazine (10 mg/kg; Alfasan International). After median laparotomy, i.p. antibiotics (metronidazole 1.5 mg/100 g BW, cefotaxime 3 mg/100 g BW) were administered and the terminal ileum was transected.

In group C, classic anastomosis, using ten interrupted, single-layer sutures (polydioxanone 7-0), was performed in the ileum of the small intestine, 10 cm away from the ileocecal valve. The rats in group S received anastomosis with TachoSil at the same area. The anastomotic line was additionally sealed

with a TachoSil<sup>TM</sup> patch ( $1.5 \times 1.5$  cm). At first, TachoSil was fixed at the mesenteric and anti-mesenteric edges, using two absorbable sutures. The posterior TachoSil was then attached to reconnect the intestine edges. At the anterior surface, the intestine edges were brought together with chromic suture, where TachoSil was then attached. The sutures were utilized to only bring the two edges together and did not play any role in anastomotic strength.

The moistened patch was applied with gentle pressure for 4–5 min while stenting the bowel with a rubber tube. The tube was removed via a small cecal incision, which was closed with two stitches (polydioxanone 7-0). Stenting was necessary, so that consistent pressure could be applied and was also used in similar large animal models.

The abdominal wall was closed with two layers of running suture (PDS 5-0). The animals were sacrificed on the tenth postoperative day, and the anastomosis site leakage and intestinal diameter were measured before and after the anastomosis.

Clinical and functional parameters included operating (anastomotic procedure) time, complication rate (death, ileus, and insufficiency), weight gain, adhesions, bursting pressure, and preanastomotic dilatation. Adhesions were quantified using a four-grade score. The isolated anastomotic segment was inflated with water to detect intraluminal bursting pressure using an inserted pressure transducer. Preanastomotic dilatation was defined as the difference between the proximal and distal luminal diameter.

The anastomoses were evaluated for the amount of inflammatory cells, fibroblasts, collagen, and neo-angiogenesis. For the histologic examination,  $4-\mu m$  longitudinal sections were stained with H&E. Modified Abramov histopathology method was used (Zekavat et al. 2016).

After providing five slices of the anastomotic site, the samples were scored as follows:

- (a) Acute inflammatory cells: boundary of the granulation tissue and inflammatory trail (score = 1).
- (b) Chronic inflammatory cells: macrophages at the early stage of granulation tissue (score = 2).
- (c) Angiogenesis: new vessel formation (score = 3).
- (d) Fibroplasia (score = 4).
- (e) Collagen: connective tissue fibroblasts (score = 5).

The highest score was assigned in the case of appearance of any of the above recovery factors. For instance, detection of the angiogenesis and fibroplasia in a sample showed that it was at the fibroplasia stage. All operations were performed by the same surgeon. The rats were kept alive for 10 days. In the animals who died before completion of the experiment, the sample was removed by laparotomy from the anastomotic site to investigate the healing status. The samples were pathologically investigated to track acute inflammatory cells, chronic inflammatory cells, neo-angiogenesis, fibroblast, and collagen, and the following results were obtained.

The obtained data were then analyzed by SPSS 20, using Chi-square test. The level of significance was set at  $p \le 0.05$ .

#### Results

The obtained results of healing status in group C reveal that healing does not proceed similarly at all stages, based on Chisquare test (p < 0.0001). These observations show that there is a difference in various stages of healing in group C.

Based on the obtained results of healing status in group S, it has been found that there is no significant difference between various healing stages in this group (p = 0.774).

The obtained results revealed that there was a statistically significant difference between the healing process in the two studied groups (p = 0.016; Table 1).

At the end of the first operation, there was no anastomosis site leakage in both groups. During the second exploration, anastomosis site leakage was observed in 83% of group S rats and 17% of group C rats, respectively (P < 0.0001). During the second exploration, more small bowel peristaltic was seen in group S compared to group C rats (P < 0.0001).

Histopathologic study revealed more acute inflammatory cells in the group S and more chronic inflammatory cells in the group C. Fibroblast proliferation and collagen formation were more in the group C. Group S had less neovascularization than group C. The control group was restored to the later stages and the case group stopped in the early stages of healing. The control group had a better healing process than the case group (Fig. 1).

In this experimental study, more acute inflammatory cells were found in the group S in comparison with the group C. This can be attributed to the continuous leakage from the anastomotic site, resulting in the death of the majority of rats due to peritonitis which had occurred in the early days. More chronic inflammatory cells were found in the group C. Neoangiogenesis occurred more often in the group S. Higher values of the fibroblast cells were detected in the group C. More collagen was produced in the group C. All of the

 Table 1
 Comparison of the healing process in groups C and S

	Group		P value
	Classic (%)	TachoSil (%)	
Granulation tissue Fibroblast	1 (5.55) 1 (5.55)	5 (27.77) 4 (22.22)	0.016
Fibroblast and collagen	4 (22.22)	6 (33.33)	
Collagen	12 (66.66)	3 (16/66)	
Total	18	18	

findings suggest that more cases in the group C proceeded to the final healing stages, and those in the group S stopped at the initial stages. Based on the microscopic examination of autopsy specimens, the following were found as the cause of death in the group S: intravascular thrombosis, acute ileitis, gangrenous bowel, microscopic peritonitis, vasculitis (fibrin deposition in the vessel wall), and hypersensitivity reaction (eosinophilia). In macroscopic observation of the samples, it was seen that TachoSil was segregated from the anastomotic area and caused severe inflammatory reaction and adhesion in all the cases in the group S.

## Discussion

Fibrin glue has been extensively used in pathology including the upper gastrointestinal tract perforations (Lau et al. 1996; Kaya et al. 2004), fistula (Buchanan et al. 2003; Zmora et al. 2003; Cintron et al. 2000; Papavramidis et al. 2004; Papavramidis et al. 2008), and anastomotic leakage (Babor et al. 2009; Silecchia et al. 2008). However, different results about the healing effect of fibrin glue have been reported. Healing process and completion of its stages are the key factors in intestinal anastomosis. Anastomotic dehiscence is seen in all of the manual, mechanical stapling, or combinational techniques (Cueto et al. 2011). TachoSil is a collagen patch coated by fibrin and thrombin coagulation factor which is employed in coronary artery anastomosis in humans (Erb et al. 2009).

In this experimental study, the control group showed a better healing process, with less complication such as abscess formation and leakage than the group S.

Nordentoft et al. could not find any difference between the healing effects of classical suture methods with suture-less methods using fibrin glue in pigs (Nordentoft et al. 2007). Karagöz et al. have compared classical anostomosis with suture-less repair using collagen patch composed of fibrin glue (Karagöz Avcı et al. 2011). They found no differences between the two methods in terms of abdominal pathological investigation, in vivo bursting pressure, and stenosis degree. They also observed that there was not any significant macroscopic difference in the healing process or infection symptoms; this finding is in the same line with the obtained results in the present study. However, there are some reports with different results from ours. Stumpf et al. compared the effects of three various types of intestinal anastomosis in 16 pigs, including standard anastomosis with suture, loose-fitting skin staple anastomosis, and suture-less anastomosis by fibrin glue. Based on their findings, a better healing pattern was macroscopically and microscopically observed in suture-less techniques (Stumpf et al. 2009). Moreover, GI secretion was reported upon using fibrin glue, by Bonanomi et al. (Bonanomi et al. 2004). Immunologic reactions to the compounds of TachoSil and the transmission of infectious diseases are the



◄ Fig. 1 a Granulation formation in classic anastomosis site suture line (× 100). b Classic anastomosis site suture line (× 40). c Fibroblast and collagen formation in classic anastomosis site (× 400). d Healing process in classic anastomosis site (× 100). e Necrotic tissue, exudate material due to anastomosis leakage (× 100). f Bowel gangrene in the classic anastomosis site (× 40). g Vasculitis, panniculitis, and fibrin deposition in the vessel wall of the TachoSil anastomosis site (× 400). h Partial vessel thrombosis in the TachoSil anastomosis site (× 400). i Panniculitis and thrombosis in the TachoSil anastomosis site (× 100). j Early collagen deposition in the group C (Masson's trichrome staining, × 400). m Scar formation in the group C (Masson's trichrome staining, × 40)

two major risks concerning topical hemostasis (Horowitz and Busch 2008).

Another risk is the transmission of infectious diseases. This is particularly dangerous for pregnant women, so the use of TachoSil is not recommended in pregnancy. TachoSil has a good satisfaction rate among the surgeons and reduces both the operating time for patients and time spent in intensive care units (Anegg et al. 2008).

## Conclusion

Based on the obtained results, TachoSil in suture-less intestinal anastomosis was not appropriate in rats and cannot be safely employed without utilizing suture. However, it may be useful to be accompanied with classic anastomosis in severe cases with high risk of leakage. Suture-less anastomosis using TachoSil is not suggested. More investigations are suggested to be performed in this field including TachoSil-suture anastomosis in future studies.

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#### **Compliance with ethical standards**

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Conflict of interest** The authors declare that they have no conflict of interest.

### References

- Anegg U, Rychlik R, Smolle-Jüttner F (2008) Do the benefits of shorter hospital stay associated with the use of fleece-bound sealing outweigh the cost of the materials? Interact Cardiovasc Thorac Surg 7(2):292–296
- Babor R, Talbot M, Tyndal A (2009) Treatment of upper gastrointestinal leaks with a removable, covered, self-expanding metallic stent.

Surgical Laparoscopy Endoscopy & Percutaneous Techniques 19(1):e1-e4

- Bhanot S, Alex JC (2002) Current applications of platelet gels in facial plastic surgery. Facial plastic surgery 18(01):027–034
- Bonanomi G, Prince J, McSteen F, Schauer P, Hamad G (2004) Sealing effect of fibrin glue on the healing of gastrointestinal anastomoses. Surg Endosc Other Interv Tech 18(11):1620–1624
- Buchanan GN, Bartram CI, Phillips RK, Gould SW, Halligan S, Rockall TA et al (2003) Efficacy of fibrin sealant in the management of complex anal fistula. Dis Colon Rectum 46(9):1167–1174
- Cintron JR, Park JJ, Orsay CP, Pearl RK, Nelson RL, Sone JH et al (2000) Repair of fistulas-in-ano using fibrin adhesive. Dis Colon Rectum 43(7):944–949
- Cueto J, Barrientos T, Rodríguez E, Del Moral P (2011) A new biodegradable adhesive for protection of intestinal anastomoses. Preliminary communication. Arch Med Res 42(6):475–481
- Erb MA, Claus T, Hartrumpf M, Bachmann S, Albes JM (2009) The use of Tachosil® surgical patch or fibrin glue in coronary artery surgery does not affect quality of anastomosis or provoke postoperative adhesions in pigs. Eur J Cardiothorac Surg 36(4):703–707
- Horowitz B, Busch M (2008) Estimating the pathogen safety of manufactured human plasma products: application to fibrin sealants and to thrombin. Transfusion. 48(8):1739–1753
- Jackson MR (2001) Fibrin sealants in surgical practice: an overview. Am J Surg 182(2):S1–S7
- Karagöz Avcı S, Yüceyar S, Aytac E, Bayraktar O, Erenler I, Ustun H, et al. (2011) Comparison of classical surgery and sutureless repair with DuraSeal or fibrin glue for duodenal perforation in rats
- Kaya C, Demir U, Coşkun H, Kalyoncu A, Gündüz B, Eroğlu T et al (2004) Comparison of repair techniques in rat duodonal perforations: simple closure, simple closure and omentoplasty, and fibrin tissue adhesive. Ulusal travma ve acil cerrahi dergisi= Turkish journal of trauma & emergency surgery: TJTES 10(1):11–16
- Lau W-Y, Leung K-L, Kwong K-H, Davey IC, Robertson C, Dawson J et al (1996) A randomized study comparing laparoscopic versus open repair of perforated peptic ulcer using suture or sutureless technique. Ann Surg 224(2):131
- Nordentoft T, Rømer J, Sørensen M (2007) Sealing of gastrointestinal anastomoses with a fibrin glue-coated collagen patch: a safety study. J Investig Surg 20(6):363–369
- Papavramidis ST, Eleftheriadis EE, Papavramidis TS, Kotzampassi KE, Gamvros OG (2004) Endoscopic management of gastrocutaneous fistula after bariatric surgery by using a fibrin sealant. Gastrointest Endosc 59(2):296–300
- Papavramidis TS, Kotzampassi K, Kotidis E, Eleftheriadis EE, Papavramidis ST (2008) Endoscopic fibrin sealing of gastrocutaneous fistulas after sleeve gastrectomy and biliopancreatic diversion with duodenal switch. J Gastroenterol Hepatol 23(12): 1802–1805
- Silecchia G, Boru CE, Mouiel J, Rossi M, Anselmino M, Morino M et al (2008) The use of fibrin sealant to prevent major complications following laparoscopic gastric bypass: results of a multicenter, randomized trial. Surg Endosc 22(11):2492–2497
- Stumpf M, Junge K, Rosch R, Krones C, Klinge U, Schumpelick V (2009) Suture-free small bowel anastomoses using collagen fleece covered with fibrin glue in pigs. J Investig Surg 22(2):138–147
- Zekavat O, Amanat A, Karami M, Paydar S, Gramizadeh B, Zareian-Jahromi M (2016) Wound healing studies using Punica granatum Peel: an animal experimental study. Adv Skin Wound Care 29(5):217–225
- Zmora O, Mizrahi N, Rotholtz N, Pikarsky AJ, Weiss EG, Nogueras JJ et al (2003) Fibrin glue sealing in the treatment of perineal fistulas. Dis Colon Rectum 46(5):584–589

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