Comparative Medicine
Copyright 2017
by the American Association for Laboratory Animal Science

Vol 67, No 6
December 2017
Pages 504–507

Original Research

Benefits of Intraluminal Agarose Stents during End-to-End Intestinal Anastomosis in New Zealand White Rabbits

Wen-Yao Kuo,[1] Hsiao-Chun Huang,[1] Shih-Wei Huang,[1] Kuan-Hua Yu,[1] Feng-Pang Cheng,[1] Jiann-Hsiung Wang,[1] and Jui-Te Wu[1,2,*]

In the present study, we evaluated the utility of an intraluminal agarose stent (IAS) for end-to-end intestinal anastomoses in rabbits. Female New Zealand white rabbits (*n* = 14) underwent conventional sutured anastomosis (CSA) with or without an IAS. IAS were used to maintain the luminal diameter for more rapid and accurate suturing, and then was squeezed transluminally to crush it into fragments, which passed through the intestines and were eliminated. The rabbits were euthanized on postoperative day 21. At necropsy, the anastomoses were assessed for adhesion formation, stenosis, and bursting pressure and were examined histologically for collagen content and blood vessel formation. Anastamosis surgery took less time in the IAS group (15.0 ± 2.6 min) than in the CSA-only group (30.1 ± 7.9 min). Only 1 postoperative death occurred (in the CSA group), and postmortem examination revealed evidence of anastomotic leakage. Adhesion formation and stenosis did not differ between groups, but bursting pressure, collagen content, and blood vessel formation were all significantly increased in the IAS group. IAS may decrease the operative time by maintaining a clear surgical field at the anastomotic site. In addition, the use of IAS promotes rapid healing and maintains the luminal diameter during end-to-end intestinal anastomosis.

Abbreviations: CSA, conventional sutured anastomosis; IAS, intraluminal agarose stent

Resection and anastomosis are commonly indicated for removing nonviable or diseased segments of the intestine. Intestinal anastomosis also is performed to treat irreducible intestinal intussusceptions.[13] Anastomotic leakage is one of the most important complications of gastrointestinal tract surgery, which reportedly occurs in 2% to 15.7% of dogs and cats.[1,3,8,12,14,15,18,21] Septic peritonitis after small intestinal surgery typically is associated with dehiscence of anastomotic or enterotomy sites. Approximately 50% of dogs die after developing intestinal dehiscence and septic peritonitis; mortality rates as high as 85% have been reported.[1,15]

Anastomotic healing is affected by both patient factors (for example, age, nutritional status, intercurrent illness) and surgical factors (for example, technique, adequate blood supply, and tension-free tissue apposition).[17,20] In addition, prolonged operative times and dirty or contaminated surgical fields are associated with increased risk of anastomotic leakage in humans and animals.[3] Alternative methods of constructing anastomoses have been investigated to reduce the operating time and level of technical expertise required to perform complex anastomoses and to increase the security of high-risk anastomoses.[19] Innovative methods for constructing anastomoses include the use of biofragmental rings and buttons, laser tissue welding, and tissue glues;[6,19] however, not every method is suitable for intestinal anastomoses in small animals.

In the present study, we evaluated the advantages of using an agarose column as an intraluminal stent, thus creating a 3D structure and maintaining precontracture luminal diameter, in conventional end-to-end intestinal anastomoses in rabbits. Adhesion formation, stenosis, and bursting pressure were assessed, and collagen content and blood vessel formation at the anastomotic site were examined histologically. We hypothesized that using an intraluminal agarose stent (IAS) can reduce the operating time and provide similar or more favorable surgical outcomes compared with conventional, sutured anastomoses (CSA) that do not incorporate IAS.

Materials and Methods

Animals. The study protocol was approved by the IACUC of National Chiayi University. Female New Zealand White rabbits (*n* = 14; age, 3 mo; mean weight, 3.0 kg) were housed in individual cages and had unrestricted access to standard rabbit chow and water. The animals alternatively underwent end-to-end CSA only (*n* = 7) or to the same CSA procedure combined with an IAS (*n* = 7).

Preparation of an intraluminal agarose stent. IAS are composed of 2% (w/v, in double-distilled water) agarose gel (Uni-Region Bio-Tech, Hsinchu, Taiwan; Figure 1). To fashion the stent, a 1-mL pipette tip was cut transversely at 3 cm from the tip and reversely inserted into another complete tip to create a double-pointed casting column. The casting column was inserted into a 15-mL centrifuge tube and steam-sterilized (121 °C for 30 min in a gravity-displacement sterilizer); the same sterilization process was used for the 2% agarose gel solution. First, 0.5 mL of sterilized agarose solution was added to the 15-mL

Received: 12 Dec 2016. Revision requested: 13 Jan 2017. Accepted: 22 Feb 2017.
[1]Department of Veterinary Medicine and [2]Animal Teaching Hospital, College of Veterinary Medicine, National Chiayi University, Chiayi City, Taiwan.
[*]Corresponding author. Email: jtwu@mail.ncyu.edu.tw

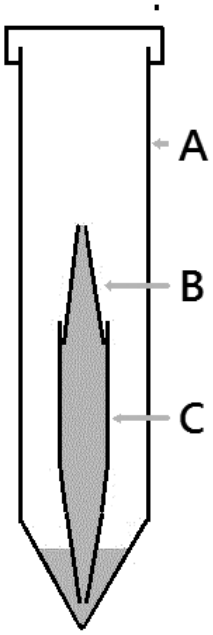

Figure 1. Preparation of an intraluminal agarose stent. To fashion the stent, a 1-mL pipette tip was transversely cut at 3 cm from the tip (B) and reversely inserted into a complete tip (C) to create a double-pointed casting column. The casting column was inserted into a 15-mL centrifuge tube (A) and steam-sterilized. The gray area indicates the sterilized 2% agarose gel.

centrifuge tube containing the casting column, and the tube was placed in a refrigerator to completely solidify the agarose gel. Then, sufficient sterilized agarose solution was added to fill the casting column and create the double-pointed agarose stent. Finally, the cap was screwed onto the tube, which then was stored at 4 °C. During surgery, the cut pipette tip was removed, and a 10-mL syringe was connected to the end of the complete tip to produce air pressure and dislodge the IAS.

End-to-end intestinal anastomosis. General anesthesia was induced in the rabbits by using intravenous propofol (6 mg/kg) and was maintained with isoflurane (2% to 5%) and oxygen by mask. No preoperative food restriction or bowel preparation was used, but rabbits received preoperative antibiotic prophylaxis (enrofloxacin, 5 mg/kg SC) and analgesia (meloxicam, 0.2 mg/kg IV) and were prepared for aseptic surgery. An abdominal midline incision was made, and the ileum was identified and then isolated by using moist gauze swabs. The marginal vessels were ligated, and the bowel was divided transversely. In the CSA-only group, the intestine was anastomosed by using 8 to 12 interrupted serosubmucosal 4-0 polydioxanone sutures (PDS II; Ethicon, Somerville, NJ). The mesenteric defect was closed in each animal, and the omentum was wrapped around the anastomotic site. Finally, the abdominal wall was closed in 2 layers by using continuous 3-0 polyglactin 910 sutures (Coated Polyglycolic Acid Suture, Ethicon).

Anastomosis in the IAS group was performed in a similar manner as for the CSA-only rabbits but included an IAS. In brief, the agarose stent was fully inserted into the orad intestinal lumen after the bowel was divided transversely. The first 2 sutures were tied at the mesenteric and antimesenteric borders, and then the IAS was advanced across the anastomosis site and was inserted into the aborad segment. Another 6 to 10 sutures were placed (Figure 2), and the IAS was massaged below the level of the anastomosis, to minimize risk of iatrogenic trauma to the anastomosis, and then crushed into fragments by

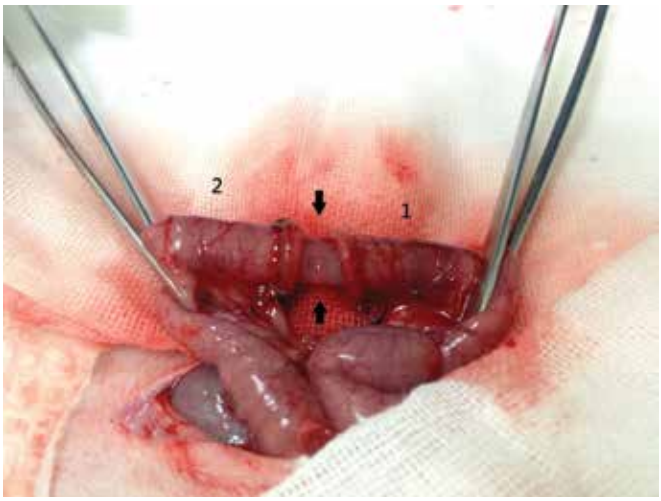

Figure 2. Using an intraluminal agarose stent during end-to-end intestinal anastomoses in rabbits. The agarose stent was fully inserted into the orad intestinal lumen (1) after the bowel was divided transversely, and then the agarose stent was advanced across the anastomosis site and was inserted into the aborad segment (2). The first 2 sutures were tied at the mesenteric and antimesenteric borders (arrow), and then 6 to 10 additional sutures were placed.

squeezing it transluminally. The duration of surgery was defined as the time from placement of the first suture at intestinal edges until the omentum wrapped around the anastomotic site.

All rabbits were allowed free access to water and food immediately after surgery, and each rabbit received meloxicam (0.1 mg/kg PO daily), ciprofloxacin (5 mg/kg PO twice daily), and metoclopramide (0.5 mg/kg PO twice daily) for 7 d.

Postoperative assessment and histologic evaluation. All rabbits were euthanized under deep anesthesia on postoperative day 21. Postmortem laparotomy was performed, and the surgeon inspected the peritoneal cavity and anastomotic area for anastomotic dehiscence and adhesion formation. Adhesion density was graded as: 0, no adhesions; 1, few perianastomotic adhesions involving the omentum only; 2, moderate perianastomotic adhesions involving the omentum or bowel loops; and 3, extensive intraperitoneal adhesions. The anastomosis was then excised centrally within a 10-cm length of bowel. The stenotic index was measured by occluding the open ends of this specimen with 3/0 silk ties, introducing a 22-gauge needle into the bowel lumen, and filling the specimen fully with air. The circumferences of the bowel 10 mm proximal and 10 mm distal to the anastomosis were then measured. The stenotic index was calculated as the ratio of the proximal to distal circumference, with a value greater than 1 indicating proximal dilation and therefore anastomotic stenosis. Subsequently, bursting pressure was measured by placing the specimen, with ends occluded and a 22-gauge needle in the bowel lumen, in a water bath. The needle was connected through a 3-way tap to a sphygmomanometer. Air was manually pumped into the lumen for 30 to 45 s until escaping air was visible in the water bath. The pressure at this point was recorded as the bursting pressure. Adhesion and stenotic indices and bursting pressure were assessed by surgeons who were not blinded to the treatment assignments.

Subsequently, the site of anastomosis was incised longitudinally along the antimesenteric border and inspected for mucosal defects and perianastomotic abscessation. After examination, longitudinal sections along both mesenteric and antimesenteric surfaces were fixed in 10% neutral buffered formalin and prepared for routine histologic evaluation after staining with

Table 1. Comparison of conventional sutured anastomosis (CSA) and CSA combined with an intraluminal agarose stent (IAS) in female New Zealand white rabbits (n = 7 per group)

	CSA	IAS	P
Survival rate (%)	86%	100%	1.08
Operative time (min)	30.1 ± 7.9	15.0 ± 2.6	0.0002
Adhesion index	1.5 ± 0.5	2.1 ± 0.9	0.078
Stenotic index	1.3 ± 0.2	1.2 ± 0.2	0.17
Bursting pressure (mm Hg)	252.5 ± 30.6	290.0 ± 9.0	0.01
Collagen content index	2.8 ± 0.4	3.6 ± 0.9	0.03
Vessel formation index	1.8 ± 0.4	2.4 ± 0.5	0.02

Data are presented as means ± 1 SD.

hematoxylin and eosin. Sections were examined by an experienced histopathologist who was blinded to the anastomotic type. Collagen content was assessed after the specimens were stained with trichrome. Collagen content and blood vessel formation at the anastomosis site were estimated on a 5-point visual scale: 0, no collagen or vessel formation; 1, areas of minimal collagen, and vessel formation in discrete areas; 2, areas of moderate collagen, and contiguous areas of vessel formation; 3, widespread collagen, and extensive areas of vessel formation within the anastomotic area; and 4, maximal collagen, and vessel formation uniformly distributed throughout the anastomotic area.[19]

Statistical analysis. The statistical analysis was performed using SPSS (version 12.0, SPSS, IL). Data are expressed as mean ± 1 SD. Unpaired t tests were used to compare the means between the 2 study groups. Grouped data were analyzed by using 2-way ANOVA, with the level of statistical significance at a P value less than 0.05.

Results

The mean duration of anastomosis surgery in our rabbits was 30.1 ± 7.9 min for the CSA-only group and 15.0 ± 2.6 min for the IAS group (P = 0.0002). During end-to-end anastomotic suturing, mucosal inversion at anastomotic sites occurred occasionally in the CAS group; conversely, mucosal eversion was present in the IAS rabbits. Once anastomosis was complete, the surgeon crushed the IAS; the agarose fragments were eliminated with feces on postoperative day 1 or 2. One animal from the CSA group died on postoperative day 4; postmortem examination revealed anastomotic leakage. The remaining 13 animals were euthanized on postoperative day 21 as planned (Table 1). Bursting pressure was higher (P = 0.001) in the IAS group than the CSA-only group, but neither adhesion formation (P = 0.078) nor stenosis (P = 0.17) differed between the groups. No anastomotic dehiscence, anastomotic obstruction, or fistula formation was found in any animal.

The mean collagen content (P = 0.003) and blood vessel formation (P = 0.002) indices were higher in the IAS group than in the CSA group, indicating that using IAS can promote collagen deposition and blood vessel formation at the anastomotic site.

Discussion

During end-to-end anastomosis, the ends of both intestinal segments frequently contract, resulting in a decreased luminal diameter and a greater challenge regarding suturing. The IAS procedure attempts to maintain the luminal diameter at the anastomotic site and thus facilitates the suturing task. This technique enabled the surgeons to perform anastomotic suturing more easily, significantly reducing the operative time (Table 1). Moreover, the agarose stent acted as an intraluminal plug and prevented abdominal contamination from intestinal contents during anastomotic suturing; this contamination is associated with an increased risk of anastomotic leakage.[15] In the current study, the surgeon easily crushed the intraluminal agarose column extraluminally by using the thumb when anastomosis was complete, and the agarose fragments were eliminated with feces on postoperative day 1 or 2. No significant postoperative complication of IAS was observed.

Compared with the CAS group, the rabbits in the IAS group had higher collagen and blood vessel formation indices and higher bursting pressure at the anastomotic site on postoperative day 21. We conclude that IAS maintain the intestinal diameter and 3D structure and might enable surgeons to more easily appose each layer of intestinal tissue during anastomotic suturing. In dogs, accurate submucosal apposition can result in primary intestinal healing, with direct bridging of the defect.[4] Accurate apposition can shorten the inflammatory phase and lead to rapid progression to the vessel development and collagen formation phases, resulting in increased bursting pressure.[7,10,11] In our preliminary study, the bursting pressure at the anastomotic site was about 30 mm Hg after an intestinal anastomosis is complete; however, the complete rabbit intestine could tolerate a bursting pressure of more than 300 mm Hg. Conversely, poor submucosal apposition results in second-intention healing, with indirect bridging of the submucosal layer and prolonged presence of an epithelial defect.[4] Furthermore, poor apposition might promote inverting anastomoses, which further cause avascular necrosis of the inverted cuff and prolongs the lag period of wound healing. Moreover, the inverted cuff increases the frequency of enteric obstruction.[2,9,11] Inverting anastomosis occasionally occurred in the CAS group.

In the current study, the omentum was sutured and wrapped around the anastomosis site to reinforce the suture line and to prevent leakage and adhesion to other abdominal tissues. Therefore, we found most of the adhered tissue at the anastomotic site was the omental membrane. The omentum has an extensive vascular and lymphatic supply and exhibits angiogenic and immunogenic properties that assist in restoring blood supply, controlling infection, and establishing lymphatic drainage.[4] However, the omentum, because of its adhesive property, can form an adherent sheath around a small-intestine anastomosis.[14] We inferred that the wrapped omentum resulted in an increased adhesion index in both the IAS and CSA groups, but the omentum sheath itself did not cause the narrowing of the intestinal lumen or increase the stenotic index. This result was consistent with that of other studies in rabbits.[16,19]

The mean adhesion index was numerically higher in the IAS group than in the CSA group, although the difference was not significant (Table 1). Interestingly, the increased adhesion formation in the IAS group did not increase the stenotic index. We found that end-to-end anastomosis with an IAS easily resulted in good apposition and only occasionally led to mild tissue eversion when the knot-tying force was excessive, thus exposing the resected wound to the wrapped omentum and further promoting adhesion formation.[5,7] Conversely, we noted tissue inversion in the CSA-only group, which reduced adhesion formation with the wrapped omentum.

In conclusion, our findings indicate a shorter operative time and likely more rapid healing at the anastomosis site when an IAS is used in conjunction with CSA for experimental intestinal anastomoses in rabbits.

References

1. **Allen DA, Smeak DD, Schertel ER.** 1992. Prevalence of small intestinal dehiscence and associated clinical factors: a retrospective study of 121 dogs. J Am Anim Hosp Assoc **28:**70–76.
2. **Ballantyne GH.** 1984. The experimental basis of intestinal suturing. Effect of surgical technique, inflammation, and infection on enteric wound healing. Dis Colon Rectum **27:**61–71.
3. **Bentley AM, Otto CM, Shofer FS.** 2007. Comparison of dogs with septic peritonitis: 1988 to 1993 versus 1999 to 2003. J Vet Emerg Crit Care **17:**391–398.
4. **Brown DC.** 2011. Small intestine, p 1513–1541. In: Tobias KM, Johnston SA, editors. Veterinary surgery: small animals, 1st ed. St Louis (MO): Saunders.
5. **Corona R, Verguts J, Schonman R, Binda MM, Mailova K, Koninckx PR.** 2011. Postoperative inflammation in the abdominal cavity increases adhesion formation in a laparoscopic mouse model. Fertil Steril **95:**1224–1228.
6. **Elemen L, Sarimurat N, Ayik B, Aydin S, Uzun H.** 2009. Is the use of cyanoacrylate in intestinal anastomosis a good and reliable alternative? J Pediatr Surg **44:**581–586.
7. **Galanopoulos G, Raptis D, Pramateftakis MG, Mantzoros I, Kanellos I, Lazarides C.** 2014. The effects of iloprost on colonic anastomotic healing in rats under obstructive ileus conditions. J Surg Res **189:**22–31.
8. **Grimes JA, Schmiedt CW, Cornell KK, Radlinksy MA.** 2011. Identification of risk factors for septic peritonitis and failure to survive following gastrointestinal surgery in dogs. J Am Vet Med Assoc **238:**486–494.
9. **Hamilton JE.** 1967. Reappraisal of open intestinal anastomoses. Ann Surg **165:**917–924.
10. **Jamieson WG, Pliagus G, Marchuk S, DeRose G, Moffat D, Stafford L, Finley RJ, Sibbald W, Taylor BM, Duff J.** 1988. Effect of antibiotic and fluid resuscitation upon survival time in experimental intestinal ischemia. Surg Gynecol Obstet **167:**103–108.
11. **Koike T, Hagio M, Yasui T, Morooka T, Sakai T.** 1976. Experimental studies in intestinal healing of the dog. IV observations on the various types of enterotomy. Jpn J Vet Res **24:**31–36.
12. **Lanz OI, Ellison GW, Bellah JR, Weichman G, VanGilder J.** 2001. Surgical treatment of septic peritonitis without abdominal drainage in 28 dogs. J Am Anim Hosp Assoc **37:**87–92.
13. **Orsher R, Rosin E.** 1993. Small intestines, p 593–612. In: Slatter D, editor. Textbook of small animal surgery. Philadelphia (PA): Saunders.
14. **Radlinsky MG.** 2013. Surgery of the digestive system: surgical techniques, p 504–515. In: Fossum TW, editor. Small animal surgery, 4th ed. St Louis (MO): Saunders.
15. **Ralphs SC, Jessen CR, Lipowitz AJ.** 2003. Risk factors for leakage following intestinal anastomosis in dogs and cats: 115 cases (1991 to 2000). J Am Vet Med Assoc **223:**73–77.
16. **Rieger N, Slaven JL, Keck J, Mackay J, Woods R.** 1999. Intestinal sleeve anastomosis: a comparative study with end-to-end anastomosis. J Surg Res **81:**170–173.
17. **Schrock TR, Devenery CW, Dunphy JE.** 1973. Factors contributing to leakage of colonic anastomoses. Ann Surg **177:**513–518.
18. **Staatz AJ, Monnet E, Seim HB 3rd.** 2002. Open peritoneal drainage versus primary closure for the treatment of septic peritonitis in dogs and cats: 42 cases (1993 to 1999). Vet Surg **31:**174–180.
19. **Sweeney T, Rayan S, Warren H, Rattner D.** 2002. Intestinal anastomoses detected with a photopolymerized hydrogel. Surgery **131:**185–189.
20. **Tagart RE.** 1981. Colorectal anastomosis: factors influencing success. J R Soc Med **74:**111–118.
21. **Wylie KB, Hosgood G.** 1994. Mortality and morbidity of small and large intestinal surgery in dogs and cats: 74 cases (1980 to 1982). J Am Anim Hosp Assoc **30:**469–474.