Experimental study of colonic anastomosis with a degradable stent in a porcine model

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Abstract

BACKGROUND: Intestinal anastomosis is a major technical component of gastrointestinal procedures. We have developed a new procedure of colonic anastomosis with a degradable stent. This article evaluates this procedure.

METHODS: Forty pigs were assigned randomly to a stent group (n = 20) and a control group (n = 20). A colonic anastomosis with a degradable stent was performed in the stent group, and hand-sewn anastomosis was performed in the control group. Pigs of each group were divided evenly into 4 subgroups according to time of death (days 3, 7, and 14, and month 10 postoperatively) to evaluate the healing of anastomosis.

RESULTS: All procedures were completed successfully. The surgical time of the stent group was significantly less than the control group. No complications occurred in either group. Bursting pressure of the stent group was significantly higher than the control group on postoperative days 3 and 7. No significant difference of hydroxyproline content or microvessel density was found between the 2 groups.

CONCLUSIONS: The procedure of colonic anastomosis with a degradable stent is a simple, feasible, and safe procedure in this porcine model.

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KEYWORDS: Colonic anastomosis; Degradable stent; Colonic surgery; Intestinal anastomosis

Intestinal anastomosis is a major technical component of gastrointestinal procedures. The hand-sewn method is the initial procedure for intestinal anastomosis and is widely used globally. Other procedures have been developed, including stapling devices, compression rings, tissue glue, and laser anastomosis. The first compression ring, Murphy button, was described in 1892, but its use was discontinued when it was discovered that its mechanical compressive pressure caused necrosis in the anastomosis site and introduced a permanent foreign body, which narrowed the intestinal lumen. Its improved version, the biofragmentable anastomosis ring, was developed in the 1980s, which partly decreased the mechanical pressure to the anastomosis site. Stapling devices facilitated anastomosis and were widely used in many countries, although these introduced unabsorbable material. Tissue glue and laser anastomosis were not used clinically because of weak anastomotic strength.

This article introduces a simple procedure for colonic anastomosis with a degradable stent. With this simple technique, suturing greatly is decreased, the anastomosis is free of compressive pressure, and the damage to the submucosal vascular plexus and mesenteric vessels are minimized. The stent is used...
as a support to resist internal pressure and longitudinal strength, thus providing higher short-term anastomotic strength. This article also evaluates the feasibility and safety of this procedure compared with hand-sewn anastomosis.

Materials and Methods

Animals

Forty experimental mini-pigs of either sex, weighing 12 to 15 kg, were housed 1 per cage at the Experimental Animal Center at Zhejiang University. They were allowed to become accustomed to the laboratory environment for more than 1 week before the start of the experiment. All animals had free access to water and standard food until the day before surgery. The study was approved by the local ethics committee of Zhejiang University.

Absorbable stent

The stent (Fig. 1) is manufactured by the Institute of Polymer Science of Zhejiang University. It is synthesized with 1,3-propanediol, 1,2-propanediol, and sebacic acid, and it then decomposes to carbon dioxide and water postoperatively. The relationship of molecular mass and degradation time in vitro is shown in Fig. 2.

Experimental design

Fifty pigs were assigned randomly to either a stent anastomosis group (SA, n = 20) or a conventional anastomosis group (CA, n = 20). The pigs were without food for 24 hours before surgery and were fed magnesium sulfate (5%) to clean the colonic lumen. Cefazolin sodium was administered intramuscularly before surgery. A colonic anastomosis by degradable stent was performed in the SA group, and hand-sewn anastomosis was performed in the CA group. The surgical time of anastomosis was recorded. Pigs of each group were divided randomly into 4 subgroups by time of death (days 3, 7, and 14, and month 10 postoperatively), and each subgroup contained 5 pigs.

In the short-term observation period, pigs were scheduled to be reoperated on postoperative days 3, 7, or 14, to evaluate the healing of anastomosis. They were killed immediately after evaluation. The peritoneal cavity was observed for signs of anastomotic leakage, stricture, colonic necrosis, volvulus, and intussusception. Approximately 5 cm of colon was resected with the anastomosis in the middle. Anastomotic strength, hydroxyproline content, and microvessel density (MVD) of anastomosis were measured. Pathology evaluation, including hematoxylin and eosin stain and trichrome Masson stain, was performed by the Pathology Department of Sir Run Run Shaw Hospital. In the long-term observation period, 5 pigs in each group were raised for 10 months postoperatively before being killed after evaluation for complications.

Procedure

Pigs were anesthetized by an intramuscular injection of ketamine and sumianxin. Laparotomy was performed via a lower-midline incision for female pigs or a lower-pararemedian incision for male pigs. At the middle of the ascending colon, a 4- to 6-cm section of colon was resected, and colonic anastomosis by stent was performed. First, the mesenteric border of the cut edge was approximated with a traction line (Fig. 3), which aided accurate tissue apposition. Polyglycolic acid sutures were used as binding lines, entering the colonic mesentery at about 5 to 10 mm to the cut edge on each side (Fig. 4A). A stent was placed in the colonic lumens with the anastomosis in the middle (Fig. 4B), and the binding lines were tied to fix the colon to the stent (Fig. 4C). Finally,
cut edges were approximated with 1 to 3 additional sutures for accurate tissue apposition (Fig. 4D). The abdomen was closed by 2 layers of interrupted sutures.

Pigs in the control group underwent a conventional, hand-sewn, end-to-end colonic anastomosis. A laparotomy was performed, and a 4- to 6-cm section of colon was resected. An end-to-end, single-layer colonic anastomosis was constructed using 15 to 20 inverting interrupted sutures. The abdomen was closed as in the SA group.

Although they were given free access to water, all pigs were without food for 24 hours postoperatively. They were given half of their normal diet on postoperative day 2. Normal diet was resumed on postoperative day 3.

Bursting pressure

Approximately 5 cm of colon with anastomosis was resected, including the surrounding tissues and adhesions, and washed in saline. Intraluminal feces were evacuated. Subsequently, one side of the anastomotic segment was connected to a manometer placed on the same plane, and the other side was closed by hemostatic forceps. The intraluminal pressure was increased gradually by an infusion of saline. Bursting pressure was defined as the maximum pressure the segment resisted or the pressure at the moment the first leakage was observed.

Hydroxyproline content

The hydroxyproline content was measured using a hydroxyproline assay kit. Tissue specimens were cleared of suture material and weighed .03 to .05 g. Specimens then were hydrolyzed in sodium hydroxide at 95°C for 20 minutes. Homogenate was diluted by adding distilled water to a total volume of 10 mL, after the pH value was adjusted to 6.0 to 6.8. Foreign matter was cleared by adding active carbon. The solution was centrifuged, and the supernatant sample was used for analysis. A total of .5 mL of chloramine-T (.05 mmol/L) was added to 1 mL of sample, and the sample was incubated at room temperature for 10 minutes. Then, 3.15 mol/L of perchloric acid was added, and the sample was incubated for 5 minutes, followed by the addition of 10% paradimethylaminobenzaldehyde. After incubation at 60°C for 15 minutes, the absorbency of the solution was measured spectrophotometrically at 550 nm and compared with standard samples for hydroxyproline content.

MVD

Immunohistochemical staining for MVD was performed with mouse antiporcine CD31 monoclonal antibody. First, frozen specimens were cut at 3 to 5 mm in a cryostat and dried at room temperature for 30 minutes. Slides were fixed by immersion in cold acetone (4°C) for 10 minutes, followed by hydration with declining concentrations of ethanol. Endogenous peroxidases were inhibited by immersion in 3% hydrogen peroxide for 10 minutes. Sections were blocked with 10% normal goat serum for 10 minutes, followed by overnight incubation at room temperature with mouse antiporcine CD31 primary antibody at a 1:300 dilution. Labeling with secondary antibody was performed using the streptavidin/peroxidase Histostain-plus Kits (ZYMED Laboratories, San Diego, CA), and visualization was achieved with the 3,3’-Diaminobenzidine Tetrahydrochloride Substrate Kit (Zhongshan Goldenbridge Biotechnology Co., Ltd, Beijing, China).

All cases were evaluated in a blinded manner. Slides first were scanned at a magnification of 40×, and 3 areas of maximum MVD (hot spots) were identified on each slide. In each of these hot spots, microvessels, such as capillaries and small venules, were counted at a magnification of 400×. A microvessel was defined as a distinct area of positive staining for CD31 (Fig. 5), whether single endothelial cells or clusters of endothelial cells, regardless of the absence or presence of a lumen. Vessels with a thick muscular layer were excluded from the count. Results were expressed as the mean number of vessels per high-power field.
Statistical analysis

For statistical analysis, independent-samples $t$ test, the Mann–Whitney $U$ test, and the Kruskal–Wallis $H$ test were used. A $P$ value of less than .05 was considered statistically significant. Statistical analysis was performed using the SPSS statistical software package (version 13.0, SPSS Inc, Chicago, IL).

Results

All cases underwent surgery successfully. The mean anastomotic time of the SA group was significantly less than the CA group ($6.7 \pm 2.7$ vs $14.8 \pm 2.7$ min; $P = .000$). There were no deaths, and no particular complications such as anastomotic leakage, stricture, colonic necrosis, volvulus, or intussusceptions were detected during the observation period.

In the short-term observation period, the bursting pressure of the SA group was significantly higher than the CA group on postoperative days 3 and 7, and it was similar on postoperative day 14 (Fig. 6). No significant differences in the hydroxyproline content and MVD were found between the SA and CA groups. Histopathologic observation of sections stained with hematoxylin and eosin or Masson’s

![Figure 4](image1.jpg)

(A) Two binding lines (a) and a traction line (b) were placed. (B) A stent was placed in the colonic lumen. (C) The colon was bound to the stent. (D) Anastomosis was completed after traction sutures were added for accurate tissue apposition.

![Figure 5](image2.jpg)

Immunohistochemical staining for microvessel density with mouse antiporcine CD31 monoclonal antibody (400X); brown-staining areas, positive staining for CD31.

![Figure 6](image3.jpg)

Bursting pressure of the SA and CA groups on day 3, $P = .009$; day 7, $P = .047$; and day 14, $P = .463$. 
trichrome stain showed that granulation tissue had formed in anastomosis on postoperative day 7, and massive thick collagen had formed on postoperative day 14 (Fig. 7).

In the long-term observation period, animals in both groups recovered uneventfully. No anorexia, marasmus, or diarrhea was seen. Stents and bind lines had disappeared at reoperation 10 months after anastomosis (Fig. 8). The anastomosis healed well, and no signs of intestinal obstruction, leakage, or anastomotic hyperplasia were evident in any case.

Comments

Hydroxyproline content is an important index in intestinal anastomosis, which reflects the formation of collagen and the healing of anastomosis. In this study, there was no significant difference in hydroxyproline content between the 2 groups. Bursting pressure is another important index for anastomatic healing, which checks the resistance to intraluminal pressure. This measure was significantly higher in the SA group than in the CA group on postoperative days 3 and 7. This result may be explained by the introduction of the stent. By using this technique, the stent occupied the peri-anastomotic colonic lumen and provided a transit for resistance to intraluminal pressure, as well as a higher bursting pressure in the SA group. Leakage is not always related to bursting pressure, but most leakage occurred because of increasing inner pressure. A higher bursting pressure indicated a potential for a lower leakage rate. No tissue splitting or necrosis occurred in the binding site, and a gradual atrophy of the intestinal wall with simultaneous hyperplasia of fibrous tissue was observed. Because intestinal atrophy is a slow process, increased collagen formation often would compensate for the gradual loss of intestinal wall and maintain intestinal integrity. In addition, a gradually increasing bursting pressure was observed in the perianastomotic co-

Figure 7  Hematoxylin and eosin (left) and Masson’s trichrome (right) stains in anastomosis of SA. (A and B) Granulation tissue formed (40×), and (C and D) collagen (400×) (stained green on slides of Masson’s stain) formed in anastomosis on postoperative day 7. (E and F) Large amounts of new thick collagen appeared on postoperative day 14 (400×).
Conclusions

This article introduces a new method of colonic anastomosis using a newly developed degradable anastomotic stent. This method is proved to be safe and feasible compared with the traditional hand-sewn method in the porcine model.

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References