Journal of Clinical and Translational Research 2022; 8(6): 453-464

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Endoluminal vacuum therapy for rectal anastomosis is safe and does not increase risk of strictures in a swine model

Alexander Ostapenko[1†], Shawn Liechty[1†], Daniel Kleiner[2*]

[1]*Department of General Surgery, Nuvance Health, CT, USA,* [2]*Department of General Surgery, Waterbury Hospital, CT, USA*
[†]*These authors contributed equally to this work*

ARTICLE INFO

Article history:
Received: March 30, 2022
Revised: May 28, 2022
Accepted: July 27, 2022
Published: October 7, 2022

Keywords:
endoluminal vacuum therapy
anastomosis
gastrointestinal leak
colorectal resection

**Corresponding author:*
Daniel Kleiner
Department of Surgery, Waterbury Hospital,
64 Robbins St, Waterbury, CT 06708, USA.
Tel: +1 203-981-2981.
Email: danieleduardkleiner@gmail.com

ABSTRACT

Background: Endoluminal vacuum therapy has been experimentally used in patients with esophageal, rectal, and Roux-en-Y bypass surgery. Yorkshire pigs are good animal models for studying the safety and efficacy of endoluminal vacuum therapy and prior studies have employed these devices in rectal anastomotic defects, as rescue therapy for early anastomotic leaks, as well as prophylactic therapy as a means of protecting high risk anastomosis.
Aim: The objective of this study is to assess the effects of a prophylactic vacuum assist device on bowel tissue surrounding an intact anastomosis at 30 days post device removal.
Methods: A total of seven pigs underwent a rectal resection with primary anastomosis: five experimental pigs with a prophylactic endoluminal vacuum device in place for 5 days post-surgery and two control pigs with no device. All animals were euthanized on the 35th post-operative day and subjected to a necropsy with a histopathological evaluation of the rectal anastomosis.
Results: No significant difference in inflammation or strictures was observed between the anastomosis of animals with the endoluminal vacuum devices and controls.
Conclusion: We, therefore, conclude that endoluminal vacuum therapy is safe for prophylactic use in pigs undergoing low anterior resection and does not cause significant strictures.
Relevance for Patients: Anastomotic leak is a feared complication resulting in increased costs, length of stay, and emotional distress. Endoluminal negative pressure vacuum therapy is a new technology that has been used in experimental models in both animals and humans for prevention and treatment of anastomotic leak. In this series we demonstrate endoluminal vacuum therapy is safe in a porcine model and does not result in stricture or increased adhesion formation. We expect endoluminal vacuum therapy to become more widely used in the future in both prevention and treatment of anastomotic leaks.

1. Introduction

Anastomotic leak is a dreaded complication in colorectal surgery carrying high morbidity and increased risk of mortality. Several studies have explored risk factors of anastomotic leaks, evaluating anatomic features, patient specific factors, operative technique, and degree of inflammation or contamination [1,2]. To reduce the risk of anastomotic leaks the American Society of Colorectal Surgery recommends both mechanical bowel preparation and oral antibiotics in all patients undergoing elective colorectal surgery [3]. Some authors argue for the use of a temporary proximal diverting ostomy as a means of reducing fecal contamination and improving the healing of a high-risk anastomosis [4]. However, this prophylactic measure subjects the patient to added discomfort and a second exposure to anesthesia and surgical procedure for the reversal of the ileostomy. A novel approach to

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

managing anastomotic leaks has been in the form of endoluminal vacuum therapy to accelerate the healing process. There have been several small studies evaluating endoluminal vacuum devices in humans for esophageal and colorectal leaks. Many studies have utilized Yorkshire pigs as animal models. Studies involving human subjects are limited but evolving.

The objective of this study was to evaluate the safety and feasibility of a prophylactic endoluminal vacuum in low anterior resection in Yorkshire pigs. Specifically, this study was conducted to assess the effects on bowel tissue surrounding an intact anastomosis at 30 days post removal of the vacuum assist closure device, after test subjects had been exposed to the device for 5 days. We hypothesized that no significant difference in anastomotic strictures or inflammation would be observed between test animals and controls.

2. Methods

2.1. Study population

Animals were housed for 14 days at CBSET facility before surgery under conditions that met or exceeded requirements as set forth in the USDA AWA/AWR. Swine were offered Purina Lab Diet (#5084 Laboratory Porcine Diet Grower) once daily, at times when animals were not on liquid diet or TPN. For bowel preparation the animals received a complete liquid diet 2 – 3 days before surgery and magnesium citrate 1 day before surgery. Animals were provided *ad libitum* access to drinking water.

2.2. Operative technique

Pigs underwent induction of anesthesia with Telazol® (4 – 6 mg/kg, IM) and isoflurane (delivered in 100% oxygen) administered through mask/nosecone. The animals were intubated and maintained on continuous inhalant isoflurane anesthesia throughout the procedure. The operative sites including the back, neck, and abdomen were clipped, prepped, and draped in the usual sterile fashion. The animals received antibiotic prophylaxis with Ceftiofur (3 – 5 mg/kg, IM) and Excede (5 mg/kg, IM).

A cut down was performed on the internal jugular vein for placement of a central venous catheter, which was tunneled subcutaneously to exit at the level of the scapula. A midline laparotomy incision measuring 6 – 10 cm was made in the lower abdomen. The descending colon and rectum were identified and mobilized ensuring the preservation of the rectal arteries. A resection was performed and the colon and rectum were anastomosed using an Ethicon curved intraluminal stapler. All anastomotic doughnuts were examined (Figure 1). After completion of the colorectal anastomosis, the endoluminal vacuum device was inserted through the anus into the rectum and its position was confirmed by manual palpation. The abdomen was lavaged with sterile saline and bupivacaine was injected for local anesthesia. The vacuum tubing was tunneled subcutaneously along the posterior aspect existing in the mid-flank to prevent dislodgment and inadvertent repositioning of the device. The device was set to 125 mm/Hg of negative pressure for 5 days.

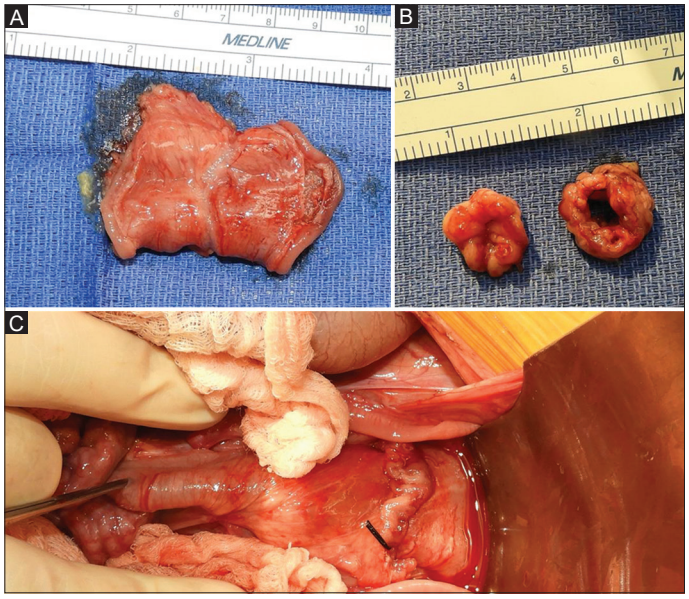

Figure 1. Representative images of rectal resection (A), anastomotic doughnuts (B), and completed anastomosis with endoluminal vacuum therapy device in place (C).

2.3. Post-operative management

Animals were placed in pocketed jackets after surgery. Test animals had portable commercial vacuum pumps in one of their jacket pockets. Animals were kept under close observation in the perioperative period and were given Carprofen (2.2 – 4.4 mg/kg, IM) for 24 – 72 h, as needed acepromazine (0.05 – 0.2 mg/kg, IV or IM) and diazepam (1 – 2 mg/kg, IV or IM) for up to the first 5 post-operative days at the discretion of the veterinary team.

Five days after surgery the endoluminal vacuum device was removed from the test animals along with the jugular venous catheter. The animals were kept on observation until post-operative day 35 at which point they underwent fluoroscopic studies to evaluate for evidence of leakage at the anastomosis. Subsequently, each animal was euthanized under anesthesia through an overdose of euthanasia solution or potassium chloride solution in accordance with accepted AVMA guidelines. A necropsy consisting of examination of the rectum, including anastomosis site, was performed on all animals to assess for leakage, dehiscence, fistula formation, and colonic stricture. The sphincter muscle complex was also assessed for damage. Macroscopic digital images of the anastomosis site were obtained at necropsy, with at least one before explant. Observations of macroscopic findings were recorded. The rectum was removed and further evaluated for anastomotic complications and injury; the anastomosis was assessed and scored for leakage, dehiscence, fistula formation, and colonic stricture. The sphincter muscle complex was also assessed for damage. The scoring system for complications and injury is reported in Tables S1 and S2 (in Supplementary File). Following evaluation, the rectum was placed in 10% NBF for histologic processing.

The rectal anastomosis site was divided into four quadrants to yield one block per quadrant. In addition, one sample 5 cm from

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

proximal tip of device/anastomosis site and one sample from 5 cm distal or 1 cm from the anus (whichever is greater) were collected as block per sample. Rectal tissues were stained with H and E for histologic evaluation. Light microscopy was used to score histological parameters that reflect the host response/repair process to the treatment. Histologic assessment was performed on sections of anastomosis site as well as sections of non-treated proximal and distal portions. The study pathologist was blinded to the treatment matrix at the time of pathological evaluation. The histopathology evaluation consisted of anastomosis integrity, tissue proliferation, necrosis, fibrosis/capsule formation, inflammation, and type and relative amounts of inflammatory cell infiltrates (e.g., neutrophils, histiocytes, lymphocytes, plasma cells, and multinucleated giant cells), any observed particulates, and evidence of micro-abscess. The full scoring metric is depicted in Table S3 (in Supplementary File).

3. Results

Of the seven pigs in this study, five underwent endoluminal negative pressure vacuum therapy for 5 days postoperatively, while two control animals did not have an endoluminal vacuum device placed. One of the experimental animals was euthanized moribund on day 3 due to progressively worsening sepsis evidenced by abdominal distension, tachycardia, and tachypnea. On necropsy the urinary bladder had a full thickness bladder tear measuring 3.5cm secondary to surgical technique. All other pigs were euthanized with Euthazol under anesthesia on the scheduled day and a necropsy was performed.

The experimental animals that underwent endoluminal negative pressure vacuum therapy did not have any local adverse local effects when compared to control animals. The anastomosis appeared to be healing well on microscopic evaluation, with no evidence of strictures at 35 days post device implantation (Table S4 in Supplementary File). Both experimental and control animals easily passed a 29 – 33 mm dilator at necropsy.

3.1. Fluoroscopy

All animals underwent fluoroscopy before necropsy, and there were no obvious leaks when filled with contrast (Figure 2). All animals had mild narrowing present at the staple line with no distinguishable difference between control and experimental animals.

3.2. Gross evaluation

All experimental animals that underwent negative pressure wound therapy had some degree adhesions around the rectal anastomosis site to the surrounding tissues. Several of the animals had some adhesions of the jejunum to the abdominal wall adjacent to the incision site. Both were secondary to the surgical procedure. The control animals did not have negative pressure wound therapy, similarly had adhesions around the anastomosis with microscopic histopathology demonstrating moderate to marked muscularis externa to adventitial/serosal segmental infiltration of neutrophils, macrophages, lymphocytes, and giant cells. Reactive fibroblasts and neovascularization were also noted at the periphery of the previously described inflamed area.

3.3. Histopathology

On histological evaluation, inflammation and inflammatory cells were comparable to slightly decreased in the experimental group when compared to control group (Figure 3). Inflammatory cells were characterized by the presence of neutrophils, histiocytes, lymphocytes, and multinucleated giant cells. The scoring is displayed in Table S3 (in Supplementary File). Inflammation scores were lower than 2 (mild) in both groups (Table S5 in Supplementary File). The mean inflammation scores for the experimental group and control group were 1.31 and 1.38, respectively. Inflammatory infiltrates were heterogeneous and were composed in both groups by neutrophils, histiocytes, lymphocytes and lesser, and rare multinucleated giant cells.

4. Discussion

Anastomotic leaks are reported to occur in 3 – 23% of cases. A number of factors have been suggested to contribute to the variable incidence including differences in demographics, surgical techniques, the use of neoadjuvant radiotherapy, diverting ostomy use, the definition of anastomotic leak, and the radiologic modality used for diagnosis [5]. Anastomotic leak can be devastating to patients and healthcare systems. A recent claims analysis of those undergoing colorectal surgery complicated by anastomotic leak found index admission costs were higher and index length of stay was longer by $30,670 and 12 days, respectively. The cost of readmission was higher and length of stay longer ($8,755 and

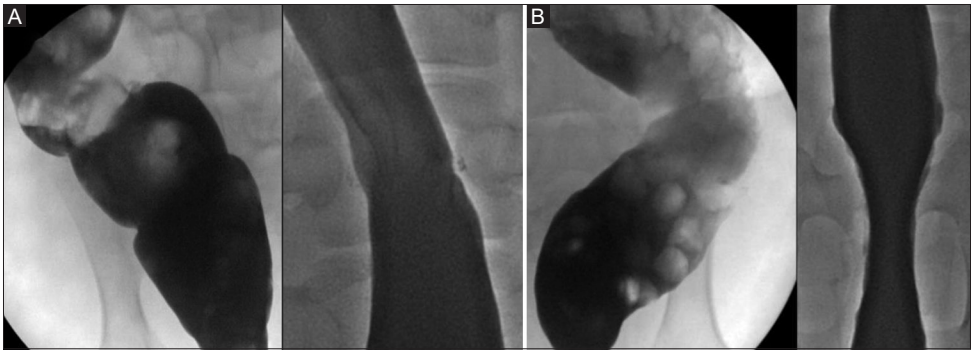

Figure 2. Representative images of fluoroscopic evaluation of anastomosis in test animals (A) and controls (B).

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

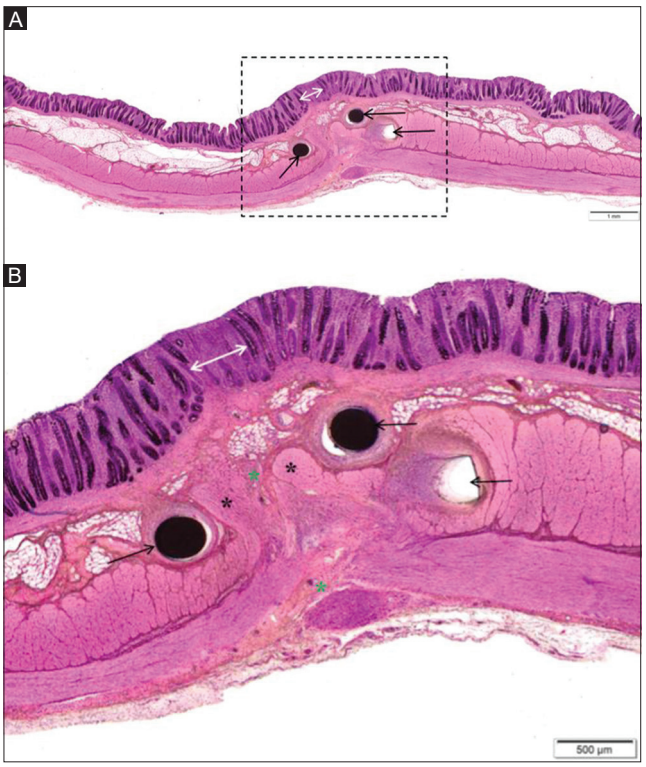

Figure 3. Representative images of histopathology of rectal anastomosis in test animal in low magnification (A) and high magnification (B). No gap is present in the anastomosis site and mucosal re-epithelialization is complete (double headed arrow). H and E stain was used.

4 days, respectively) for patients with an anastomotic leak [6]. Many efforts have been made to reduce anastomotic leaks, including preoperative bowel prep, intraoperative leak test, anastomotic buttress, endoscopic evaluation, and perfusion assessment. Some groups have shown successful reductions in anastomotic leak rates by creating a proximal diverting ostomy. In a meta-analysis containing randomized controlled trials and comparative analyses, proximal diversion resulted in decreased risk of anastomotic leak (RR = 0.43, 95% confidence interval [CI] = 0.28 – 0.67) and reoperations (RR = 0.62, 95% CI = 0.40 – 0.90). Diversion, however, resulted in increased morbidity (RR = 1.32, 95% CI = 1.05-1.65) [7]. In addition, patients with proximal diversion will require reversal in the future. The costs of morbidity and reversal can be high, with one study citing as much as $43,000 more per patient [8]. Current practices successfully reduce the morbidity of anastomotic leak, but result in increased costs and future operative interventions. For these reasons, we sought to investigate a novel, prophylactic endoluminal vacuum assisted device.

International groups have published promising results in treating esophagogastric and colorectal anastomotic leaks in human subjects, but systematic reviews show considerable variability due to differences in definitions of success, device assembly, and placement [9-12]. Further, indications for vacuum therapy are not well defined. Lehwald-Tywuschik *et al.* recently published a prospective observational study in a cohort of 14 patients evaluating prophylactic endoluminal vacuum therapy for a variety of conditions. They found a 92% success rate with gastrointestinal continuity preserved in all cases. The study, however, had significant limitations including low sample size, heterogeneity of indications, and risk of failure without a diverting stoma [13] In addition, there is no histopathologic or radiographic evaluation before or after vacuum therapy. Another group published a case report of a successfully managed anastomotic leak, which was treated with endoluminal vacuum therapy as a prophylactic measure after redo-surgery. This single center case report is difficult to apply generally given its anecdotal evidence [14]. Popivanov *et al.* published the largest series to date, a systematic review analyzing 295 cases of endoluminal negative pressure therapy for colorectal anastomotic leaks. Interestingly, the only statistically significant factor in predicting vacuum therapy success was the presence of a diverting stoma [15]. The current data does not delineate the combined or individual effects associated with diverting stoma, endoluminal vacuum therapy, or both. The current literature is confounded by a variety of indications for therapy including malignant and benign disease, subjective inclusion criteria, mixed device use (prophylactic and therapeutic during index and redo surgery), and a lack of radiographic or histopathologic evaluation.

For these reasons, we designed this study to assess feasibility and safety with standardized specifications for device assembly and placement in a swine model. There was no evidence of stenosis on pre-necropsy fluoroscopy or necropsy gross evaluation. The endoluminal VAC did not result in increased adhesion formation, and microscopic examination showed evidence of healing without abscess or mucosal disruption. These results suggest endoluminal VAC therapy does not increase risk of stricture or adhesion formation and is safe to use. We expect this novel therapy to become a common treatment modality for patients with anastomosis at risk for leak. Further studies are needed to delineate specific indications for therapy and the effect of confounding therapies on success.

The unique nature of our study shows endoluminal vacuum therapy is safe and feasible by histopathologic and radiographic data. One of the main limitations of our study is that the sample size is small. However, this is consistent with other studies in the literature that report on outcomes with endoluminal devices for both human patients and animal models. Due to the paucity of data an available literature studies that contribute to the literature play an important role in this rapidly developing field. Endoluminal vacuum therapy has a potential to reduce morbidity for many high risk operations and assist in managing surgical complications.

5. Conclusion

Endoluminal negative pressure therapy is safe and feasible for rectal anastomosis in porcine animal model. No local adverse effects were observed in test animals compared to controls thirty-5 days after device implantation. Specifically no significant differences in inflammation were observed on microscopic and macroscopic evaluation between the test group and control group. Furthermore, endoluminal negative pressure therapy did not cause

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

stricture formation at the anastomosis when compared to control animals. Further evaluation of endoluminal vacuum therapy in human clinical trials is warranted.

Acknowledgments

None.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors declare no conflict of interest

References

[1] Telem DA, Chin EH, Nguyen SQ, Divino CM. Risk Factors for Anastomotic Leak Following Colorectal Surgery: A Case-control Study. Arch Surg 2010;145:371-6.

[2] Eberl T, Jagoditsch M, Klingler A, Tschmelitsch J. Risk Factors for Anastomotic Leakage after Resection for Rectal Cancer. Am J Surg 2008;196:592-8.

[3] Migaly J, Bafford AC, Francone TD, Gaertner WB, Eskicioglu C, Bordeianou L, *et al*. The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the Use of Bowel Preparation in Elective Colon and Rectal Surgery. Dis Colon Rectum 2019;62:3-8.

[4] Blumetti J, Abcarian H. Management of Low Colorectal Anastomotic Leak: Preserving the Anastomosis. World J Gastrointest Surg 2015;7:378.

[5] You YN, Hardiman KM, Bafford A, Poylin V, Francone TD, Davis K, *et al*. The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the Management of Rectal Cancer. Dis Colon Rectum 2020;63:1191-222.

[6] Lee SW, Gregory D, Cool CL. Clinical and Economic Burden of Colorectal and Bariatric Anastomotic Leaks. Surg Endosc 2020;34:4374-81.

[7] Pisarska M, Gajewska N, Małczak P, Wysocki M, Witowski J, Torbicz G, *et al*. Defunctioning Ileostomy Reduces Leakage Rate in Rectal Cancer Surgery-systematic Review and Meta-analysis. Oncotarget 2018;9:20816.

[8] Stey AM, Brook RH, Keeler E, Harris MT, Heimann T, Steinhagen RM. Outcomes and Cost of Diverted Versus Undiverted Restorative Proctocolectomy. J Gastrointest Surg 2014;18:995-1002.

[9] Rubicondo C, Lovece A, Pinelli D, Indriolo A, Lucianetti A, Colledan M. Endoluminal Vacuum-assisted Closure (E-Vac) Therapy for Postoperative Esophageal Fistula: Successful Case Series and Literature Review. World J Surg Oncol 2020;18:1-7.

[10] Smallwood NR, Fleshman JW, Leeds SG, Burdick J. The Use of Endoluminal Vacuum (E-Vac) Therapy in the Management of Upper Gastrointestinal Leaks and Perforations. Surg Endosc 2016;30:2473-80.

[11] Bludau M, Hölscher A, Herbold T, Leers J, Gutschow C, Fuchs H, *et al*. Management of Upper Intestinal Leaks Using an Endoscopic Vacuum-assisted Closure System (E-VAC). Surg Endosc 2014;28:896-901.

[12] Sharp G, Steffens D, Koh CE. Evidence of Negative Pressure Therapy for Anastomotic Leak: A Systematic Review. ANZ J Surg 2021;91:537-45.

[13] Lehwald-Tywuschik NC, Alexander A, Alkhanji N, Flügen G, Fung S, Rehders A, *et al*. The "Impossible" Rectal Anastomosis: A Novel Use for Endoluminal Vacuum-assisted Therapy. Tech Coloproctol 2021;25:125-30.

[14] Mandarino F, Barchi A, Biamonte P, Esposito D, Azzolini F, Fanti L, *et al*. The Prophylactic Use of Endoscopic Vacuum Therapy for Anastomotic Dehiscence after Rectal Anterior Resection: Is it Feasible for Redo Surgery? Tech Coloproctol 2022;26:319-20.

[15] Popivanov G, Mutafchiyski V, Cirocchi R, Chipeva S, Vasilev V, Kjossev KT, *et al*. Endoluminal Negative Pressure Therapy in Colorectal Anastomotic Leaks. Colorectal Dis 2020;22:243-53.

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

Journal of Clinical and Translational Research 2022; 8(6): 453-464

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

Endoluminal vacuum therapy for rectal anastomosis is safe and does not increase risk of strictures in a swine model

Supplementary File

Table S1. Macroscopic scoring for anastomotic complications

Score	Observation
0	No adhesions or abnormalities
1	Adhesion to fat pad, clean anastomosis underneath
2	Adhesion to intestinal loop, abdominal wall or other organ
3	Anastomotic defect found underneath adhesion, no other abnormalities
4	Signs of possible contamination (e.g., small abscesses)
5	Clear anastomotic complication; spread of pus, obstruction at anastomosis, sign of peritonitis
6	Fecal peritonitis/death due to peritonitis

Table S2. Macroscopic scoring for bowel injury.

Score	Observation
1	Contusion/hematoma/laceration-partial thickness, no perforation
2	Full thickness defect <50% of circumference
3	Full thickness defect >50% of circumference without transection

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

Table S3. Microscopic changes scoring matrix.

Score	Anastomosis integrity
0	Intact, no defect
1	Defect restricted to mucosa/submucosa
2	Defect extends to muscularis externa
3	Defect extends to adventitia
4	Regional peritoneal involvement
Score	**Tissue proliferation**
0	Absent
1	Minimal, immature tissue restricted to edges of anastomosis
2	Mild with involvement of <50% of anastomosis
3	Mild with involvement of >50% of anastomosis
4	Marked, diffuse tissue repair and maturation
Score	**Inflammation**
0	Absent
1	Rare, minimal, ~1–5/per high power field (hpf; 40× obj)
2	Mild, multifocal or locally extensive ~5–10/hpf
3	Moderate confluent infiltrate with preservation of normal tissue architecture
4	Packed, overwhelming infiltrate with effacement of regional architecture
Score	**Necrosis**
0	Absent
1	Minimal, focal, nearly imperceptible
2	Mild, focally extensive, inconspicuous
3	Moderate, multifocal or locally extensive, readily apparent
4	Severe, regionally extensive, overwhelming with effacement of regional architecture
Score	**Fibrosis**
0	Absent
1	Minimal, e.g., narrow band, ~1–2 cell layers thick
2	Mild, e.g., thin, localized band, <~10 cell layers thick
3	Moderately thick, contiguous band along length of tissue/ structure
4	Extensive zone with regional extension effacement of local architecture

Table S4. Macroscopic evaluation of experimental and control animals at necropsy.

Macroscopic observations at necropsy and their correlating microscopic interpretation

Time point	Group	Treatment	Animal number	Anastomosis complications score	Bowel Injury score	Largest diameter dilator passing through anastomosis (mm)	Tissue/ organ	Macroscopic observation	Microscopic observation	Macro-micro correlation"
	Group 1		52851	0	3	33	Rectal anastomosis	Minor to minimal adhesion around anastomosis site, required some cutting.	Presence of mucosal glands on the serosal surface with inflammation due to anastomosis procedure.	Yes
							Small intestine	Adhesion, jejunum to abdomen wall at surgery incision site, broke away easily with finger dissection.	NA	
							Thymus	Enlarged, ~ 4 x 6 cm by ~ 18 mm thick.	Slightly increased cortical cellularity with maintained architecture.	Yes

(Contd...)

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

Table S4. *(Continued).*

Macroscopic observations at necropsy and their correlating microscopic interpretation

Time point	Group	Treatment	Animal number	Anastomosis complications score	Bowel lnjury score	Largest diameter dilator passing through anastomosis (mm)	Tissue/ organ	Macroscopic observation	Microscopic observation	Macro-micro correlation"
							Skin	Subcutaneous, accumulation, purulent liquid, grayish/greenish/ yellow. Did not have a pungent smell. ~ 5 x 8 cm by ~ 32 mm in height.	NA	
			52981	0	0	29	No Abnormal Findings.			
			52982	0	0	29	Surgery incision site	Abdominal hernia ~ 6 x 7 cm, small amount of jejunum adhered to distal/caudal end of hernia.	NA	
			53184	0	NA	33	Abdomen incision	Adhesion, fibrous, jejunum to umbilicus/abdomen incision, firm.	NA	
							Rectum/ rectal anastomosis	A fibrinous formation/ accumulation on the lateral surfaces of the colon/rectum exceeding ~ 10-12 cm cranial to the anastomosis and ~ 3-3.5 cm caudal to site. Did not appear to inhibit intestinal function.	A fibrous tissue band is present on the adventitial surface and within the adipose tissue immediately adjacent to the adventitia.	Yes
	Group 2		52852	1	3	33	Rectal anastomosis	Mild adhesion to uterus and ureters (bilateral), required cutting, right ovary adhered to colon ~ 10 cm from anastomosis site. Firm, nodule, purulent liquid, grayish/greenish/ yellow, did not have a pungent smell, diameter ~ 20 mm.	Moderate to marked muscularis externa to adventitial segmental infiltration of neutrophils, macrophages lymphocytes and giant cells with rare small foreign material particles. Reactive fibroblasts and neovascularization also noted at the periphery of inflamed area.	Yes
							Skin	Surgical incision site, hernia ~ 45 mm opening, partially healed/closed. Able to see anastomosis clips/ staples on the mucosal surface of intestine.	NA	

(Contd...)

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

Table S4. *(Continued).*

Macroscopic observations at necropsy and their correlating microscopic interpretation

Time point	Group	Treatment	Animal number	Anastomosis complications score	Bowel lnjury score	Largest diameter dilator passing through anastomosis (mm)	Tissue/ organ	Macroscopic observation	Microscopic observation	Macro-micro correlation"
			52949	1	3	22	Kidneys	Bilateral renal enlargement: Moderate left (13.0 x 6.0 x 5.0 cm); Marked right (15.0 x 6.0 x 6.0 cm). Bilateral pelvic/ cystic dilatation with cortical atrophy (right greater than left).	Moderate to marked bilateral cystic pelvis with bilateral atrophy of adjacent renal parenchyma. Mild interstitial fibrosis with mild inflammation (mainly mononuclear) with minimal intrapelvic hemorrhage are also present in the left kidney.	Yes
							Ureters	Marked dilatation of proximal ureters (1.0 cm diameter right; 1.2 cm diameter left). Distal ureters (at trigone) within normal limits/0.4 cm diameter.	No abnormal histological findings.	No
							Small intestine	Adhesion, small intestine (jejunum) to incision, firmly adhered, small omental adhesion to umbilicus to liver, loosely adhered.	NA	

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

Table 5. Histopathological evaluation of experimental and control animals.

Planar, site, and group histomorphology data						Inflammation	Neutrophils	Histiocytes	Lymphocytes	Plasma cells	Multinucleated giant cells	Particulates
Time point	Group	Treatment	Animal number	Tissue	Site							
Day 35	Group 1	Vacuum	52851	Rectum	NP	0	0	0	0	0	0	0
				Anastomosis	1	2	1	2	1	0	0	0
					2	1	1	1	1	0	0	0
					3	1	0	1	1	0	0	0
					4	2	1	1	1	0	1	1
				Rectum	ND	0	0	0	0	0	0	0
				Anastomosis	Mean	1.50	0.75	1.25	1.00	0.00	0.25	0.25
			52981	Rectum	NP	0	0	0	0	0	0	0
				Anastomosis	1	2	1	1	1	0	0	0
					2	1	1	1	1	0	0	0
					3	2	1	1	1	0	0	0
					4	1	1	1	1	0	0	0
				Rectum	ND	0	0	0	0	0	0	0
				Anastomosis	Mean	1.50	1.00	1.00	1.00	0.00	0.00	0.00
Day 35	Group 1	Vacuum	52982	Rectum	NP	0	0	0	0	0	0	0
				Anastomosis	1	2	1	2	1	0	0	0
					2	1	1	1	1	0	0	0
					3	1	1	1	1	0	0	0
					4	1	1	1	1	0	0	0
				Rectum	ND	0	0	0	0	0	0	0
				Anastomosis	Mean	1.25	1.00	1.25	1.00	0.00	0.00	0.00
			53184	Rectum	NP	0	0	0	0	0	0	0
				Anastomosis	1	1	0	1	1	0	0	0
					2	1	1	1	1	0	0	0
					3	1	0	1	1	0	0	0
					4	1	1	1	1	0	0	0
				Rectum	ND	0	0	0	0	0	0	0
				Anastomosis	Mean	1.00	0.50	1.00	1.00	0.00	0.00	0.00
Day 35: Group 1 - Vacuum (n=4)					Mean	1.31	0.81	1.13	1.00	0.00	0.06	0.06
					SD	0.24	0.24	0.14	0.00	0.00	0.13	0.13
					Median	1.38	0.88	1.13	1.00	0.00	0.00	0.00
					Incidence	100%	100%	100%	100%	0%	25%	25%
Day 35	Group 2	No Vacuum	52852	Rectum	NP	0	0	0	0	0	0	0
				Anastomosis	1	2	2	1	1	0	1	0
					2	1	1	1	1	0	1	0
					3	1	1	1	1	0	1	0
					4	2	2	2	1	0	0	0
				Rectum	ND	0	0	0	0	0	0	0
				Anastomosis	Mean	1.50	1.50	1.25	1.00	0.00	0.75	0.00

(Contd...)

Table 5. *(Continued).*

Planar, site, and group histomorphology data						Inflammation	Neutrophils	Histiocytes	Lymphocytes	Plasma cells	Multinucleated giant cells	Particulates
Time point	Group	Treatment	Animal number	Tissue	Site							
			52949	Rectum	NP	0	0	0	0	0	0	0
				Anastomosis	1	1	1	1	1	0	0	0
					2	1	1	1	1	0	0	1
					3	1	1	1	1	0	0	0
					4	2	1	1	1	0	0	0
				Rectum	ND	0	0	0	0	0	0	0
				Anastomosis	Mean	1.25	1.00	1.00	1.00	0.00	0.00	0.25
Day 35: Group 2 - No Vacuum (n=2)					Mean SD	1.38	1.25	1.13	1.00	0.00	0.38	0.13
					Median	0.18	0.35	0.18	0.00	0.00	0.53	0.18
					Incidence	1.38	1.25	1.13	1.00	0.00	0.38	0.13
						100%	100%	100%	100%	0%	50%	50%

Planar, site, and group histomorphology data						Micro-abscess	Anastomosis integrity	Tissue proliferation	Necrosis	Fibrosis/ capsule formation	Comments/ observations
Time point	Group	Treatment	Animal number	Tissue	Site						
Day 35	Group 1	Vacuum	52851	Rectum	NP	0	NA	0	0	0	None.
				Anastomosis	1	0	0	4	0	2	Presence of mucosal glands on the serosal surface with inflammation due to anastomosis procedure. Staples with surrounding inflammation are present. Lymphoid follicles present within submucosa.
					2	0	0	4	0	1	Staples with surrounding inflammation are present.
					3	0	0	4	0	2	Staple with surrounding inflammation is present.
					4	0	0	4	0	2	Staple with surrounding inflammation is present. Particulate is surrounded by granulomatous inflammation.
				Rectum	ND	0	NA	0	0	0	None.
				Anastomosis	Mean	0.00	0.00	4.00	0.00	1.75	
			52981	Rectum	NP	0	NA	0	0	0	None.
				Anastomosis	1	0	0	4	0	2	Staples with surrounding inflammation are present.
					2	0	0	4	0	2	Staples with surrounding inflammation are present.
					3	0	0	4	0	3	Staples with surrounding inflammation are present. Anastomosis site filled by adipose tissue, fibro-vascular proliferation and inflammation. Lymph node section present on adventitia.
					4	0	0	4	0	2	Staples with surrounding inflammation are present.
				Rectum	ND	0	NA	0	0	0	None.
				Anastomosis	Mean	0.00	0.00	4.00	0.00	2.25	
Day 35	Group 1	Vacuum	52982	Rectum	NP	0	NA	0	0	0	None.
				Anastomosis	1	0	0	4	0	2	Staples with surrounding inflammation are present.
					2	0	0	4	0	2	Staples with surrounding inflammation are present. Presence of mucosal glands on the serosal surface with inflammation due to anastomosis procedure.

(Contd...)

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

DOI: http://dx.doi.org/10.18053/jctres.08.202206.001

Table 5. *(Continued).*

Planar, site, and group histomorphology data						Micro-abscess	Anastomosis integrity	Tissue proliferation	Necrosis	Fibrosis/ capsule formation	Comments/ observations
Time point	Group	Treatment	Animal number	Tissue	Site						
					3	0	0	4	0	2	Staples with surrounding inflammation are present. Presence o dilated/cystic mucosal gland/s on the serosal surface with inflammation due to anastomosis procedure.
					4	0	0	4	0	2	Staples with surrounding inflammation are present.
				Rectum	ND	0	NA	0	0	0	None.
				Anastomosis	Mean	0.00	0.00	4.00	0.00	2.00	
			53184	Rectum	NP	0	NA	0	0	0	None.
				Anastomosis	1	0	0	4	0	2	Staples with surrounding inflammation are present.
					2	0	0	4	0	2	Staples with surrounding inflammation are present.
					3	0	0	4	0	2	Staples with surrounding inflammation are present.
					4	0	0	4	0	2	Staples with surrounding inflammation are present.
				Rectum	ND	0	NA	0	0	0	None.
				Anastomosis	Mean	0.00	0.00	4.00	0.00	2.00	
Day 35: Group 1 - Vacuum (n=4)					Mean	0.00	0.00	4.00	0.00	2.00	
					SD	0.00	0.00	0.00	0.00	0.20	
					Median	0.00	0.00	4.00	0.00	2.00	
					Incidence	0%	0%	100%	0%	100%	
Day 35	Group 2	No Vacuum	52852	Rectum	NP	0	NA	0	0	0	None.
				Anastomosis	1	0	0	4	0	2	Presence of mucosal glands on the serosal surface with inflammation due to anastomosis procedure. Staples with surrounding inflammation are present.
					2	0	0	4	0	2	Staples with surrounding inflammation are present.
					3	0	0	4	0	2	Staples with surrounding inflammation are present.
					4	0	0	4	0	2	Staples with surrounding inflammation are present.
				Rectum	ND	0	NA	0	0	0	None.
				Anastomosis	Mean	0.00	0.00	4.00	0.00	2.00	
			52949	Rectum	NP	0	NA	0	0	0	Indentation noted within mucosa with inflammatory infiltrate likely to be preexisting background finding or part of anastomosis site inadvertently captured in the section.
				Anastomosis	1	0	0	4	0	1	Staples with surrounding inflammation are present.
					2	0	0	4	0	1	Staple with surrounding inflammation is present. Presence of mucosal glands on the serosal surface with inflammation due to anastomosis procedure.
					3	0	0	4	0	2	Staple with surrounding inflammation is present. Mucosal pseudo diverticulum formation with ingesta and debris accumulation.
					4	0	0	4	0	2	Staples with surrounding inflammation are present.
				Rectum	ND	0	NA	0	0	0	Lymphoid follicles present within submucosa.
				Anastomosis	Mean	0.00	0.00	4.00	0.00	1.50	
Day 35: Group 2 - No Vacuum (n=2)					Mean SD	0.00	0.00	4.00	0.00	1.75	
					Median	0.00	0.00	0.00	0.00	0.35	
					Incidence	0.00	0.00	4.00	0.00	1.75	
						0%	0%	100%	0%	100%	