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

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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 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.

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

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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.

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
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
assessment 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).
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 stenosis 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-five 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

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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 esophageal 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-Twyuschik et al. recently published a prospective observational study in a cohort of 14 patients evaluating prophylactic endoluminal vacuum therapy.
striction formation at the anastomosis when compared to control animals. Further evaluation of endoluminal vacuum therapy in human clinical trials is warranted.

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**Conflict of Interest**

The authors declare no conflict of interest

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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 |
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; 40x 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 | Rectal anastomosis | 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...)
| 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 |
|------------|-------|-----------|---------------|-------------------------------|-------------------|-----------------------------------------------------------|-------------|-------------------------|------------------------|-------------------------|
|            |       |           |               |                               |                   |                                                           | 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. | 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 materials 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...)
Table S4. (Continued).

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 |
|------------|-------|-----------|---------------|---------------------------------|-------------------|----------------------------------------------------------|--------------|------------------------|------------------------|-------------------------|
|            | 52949 |           | 1             | 3                              | 22                | 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). | Kidneys      | 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         |            |
|            |       |           |               |                                 |                   | 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. | Ureters      | No abnormal histological findings. | No          |            |
|            |       |           |               |                                 |                   | Adhesion, small intestine (jejunum) to incision, firmly adhered, small omental adhesion to umbilicus to liver, loosely adhered. | Small intestine | NA                     |            |            |
Table 5. Histopathological evaluation of experimental and control animals.

| Time point | Group | Treatment | Animal number | Tissue Site | Planar | Animal | Inflammation | Neutrophils | Histiocytes | Lymphocytes | Plasma cells | Multinucleated giant cells | Particulates |
|------------|-------|-----------|---------------|-------------|--------|--------|-------------|-------------|-------------|-------------|--------------|--------------------------|-------------|
| Day 35     | Group 1 | Vacuum    | 52851         | Rectum NP   | 0      | 0      | 0           | 0           | 0           | 0           | 0            | 0            | 0             |
| 1          | Anastomosis | 2       | 1            | 2           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| 2          | Anastomosis | 1       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| 3          | Anastomosis | 1       | 0            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| 4          | Anastomosis | 2       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| Rectum     | ND     | 0         | 0            | 0           | 0      | 0      | 0           | 0           | 0           | 0           | 0            | 0            |
| Anastomosis Mean | 1.50 | 0.75 | 1.25 | 1.00 | 0.00 | 0.25 | 0.25 |
| Day 35     | Group 1 | Vacuum    | 52981         | Rectum NP   | 0      | 0      | 0           | 0           | 0           | 0           | 0            | 0            | 0             |
| 1          | Anastomosis | 2       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| 2          | Anastomosis | 1       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| 3          | Anastomosis | 2       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| 4          | Anastomosis | 1       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| Rectum     | ND     | 0         | 0            | 0           | 0      | 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    | 53184         | Rectum NP   | 0      | 0      | 0           | 0           | 0           | 0           | 0            | 0            | 0             |
| 1          | Anastomosis | 2       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| 2          | Anastomosis | 1       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| 3          | Anastomosis | 2       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| 4          | Anastomosis | 1       | 1            | 1           | 1      | 1      | 0           | 0           | 0           | 0           | 0            | 0            |
| Rectum     | ND     | 0         | 0            | 0           | 0      | 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 (n=4)

|               | SD   | Median | Incidence | SD | Median | Incidence |
|---------------|------|--------|-----------|----|--------|-----------|
| Mean          | 0.24 | 1.38   | 100%      | 0.24 | 0.88 | 100%      |
| Mean          | 0.48 | 1.13   | 100%      | 0.14 | 0.13 | 100%      |
| Incidence     | 100% | 100%   | 100%      | 100%| 100% | 100%      |
| 0%            | 0%   | 0%     | 0%        | 0%  | 0%    | 0%        |
| 25%           | 25%  | 25%    | 25%       | 25% | 25%   | 25%       |

Day 35: Group 2 - No Vacuum

|               | SD   | Median | Incidence | SD | Median | Incidence |
|---------------|------|--------|-----------|----|--------|-----------|
| Mean          | 0.24 | 1.38   | 100%      | 0.24 | 0.88 | 100%      |
| Mean          | 0.48 | 1.13   | 100%      | 0.14 | 0.13 | 100%      |
| Incidence     | 100% | 100%   | 100%      | 100%| 100% | 100%      |
| 0%            | 0%   | 0%     | 0%        | 0%  | 0%    | 0%        |
| 25%           | 25%  | 25%    | 25%       | 25% | 25%   | 25%       |

(Contd...)
Planar, site, and group histomorphology data (Continued).

| Time point | Group | Treatment | Animal number | Tissue | Site | Inflammation | Neutrophils | Histiocytes | Lymphocytes | Plasma cells | Multinucleated giant cells | Particulates |
|------------|-------|-----------|---------------|--------|------|--------------|-------------|-------------|-------------|--------------|-----------------------------|-------------|
| 52849      | Rectum| NP        | 0             | 0      | 0    | 0            | 0           | 0           | 0           | 0            | 0                           | 0           |
| Anastomosis|       | 1         | 1             | 1      | 1    | 1            | 0           | 0           | 0           | 0            | 0                           | 0           |
|            |       | 2         | 1             | 1      | 1    | 1            | 0           | 0           | 0           | 0            | 1                           | 0           |
|            |       | 3         | 1             | 1      | 1    | 1            | 0           | 0           | 0           | 0            | 0                           | 0           |
|            |       | 4         | 2             | 1      | 1    | 1            | 0           | 0           | 0           | 0            | 0                           | 0           |
| Rectum     | NP    | 0         | 0             | 0      | 0    | 0            | 0           | 0           | 0           | 0            | 0                           | 0           |
| Anastomosis|       | 1         | 1.25          | 1.00   | 1.00 | 1.00         | 0.00        | 0.00        | 0.00        | 0.00         | 0.25                        | 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

| Time point | Group | Treatment | Animal number | Tissue | Site | Micro-abscess integrity | Tissue proliferation | Necrosis | Fibrosis/ capsule formation | Comments/ observations |
|------------|-------|-----------|---------------|--------|------|-------------------------|--------------------|----------|-----------------------------|------------------------|
| Day 35     | Group 1| Vacuum    | 52851         | Rectum| NP   | 0                       | NA                 | 0        | 0                           | None.                  |
| Anastomosis|       | 1         | 0             | 0      | 4    | 0                       | 0                  | 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                       | 0                  | 0        | 1                           | Staples with surrounding inflammation are present. |
|            |       | 3         | 0             | 0      | 4    | 0                       | 0                  | 0        | 2                           | Staple with surrounding inflammation is present. |
|            |       | 4         | 0             | 0      | 4    | 0                       | 0                  | 0        | 2                           | Staple with surrounding inflammation is present. Particulate is surrounded by granulomatous inflammation. |
| Rectum     | NP    | 0         | NA            | 0      | 0    | 0                       | 0                  | 0        | None.                       | None.                  |
| Anastomosis| Mean  | 0.00      | 0.00          | 4.00   | 0.00 | 1.75                    |                    |          |                             |                         |
| 52981      | Rectum| NP        | 0             | NA     | 0    | 0                       | 0                  | 0        | None.                       | Staples with surrounding inflammation are present. |
| Anastomosis|       | 1         | 0             | 0      | 4    | 0                       | 0                  | 0        | 2                           | Staples with surrounding inflammation are present. |
|            |       | 2         | 0             | 0      | 4    | 0                       | 0                  | 0        | 2                           | Staples with surrounding inflammation are present. |
|            |       | 3         | 0             | 0      | 4    | 0                       | 0                  | 0        | 2                           | Staples with surrounding inflammation are present. Anastomosis site filled by adipose tissue, fibrovascular proliferation and inflammation. Lymph node section present on adventitia. |
|            |       | 4         | 0             | 0      | 4    | 0                       | 0                  | 0        | 2                           | Staples with surrounding inflammation are present. |
| Rectum     | ND    | 0         | NA            | 0      | 0    | 0                       | 0                  | 0        | None.                       | 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                           | None.                  |
| Anastomosis|       | 1         | 0             | 0      | 4    | 0                       | 0                  | 0        | 2                           | Staples with surrounding inflammation are present. |
|            |       | 2         | 0             | 0      | 4    | 0                       | 0                  | 0        | 2                           | Staples with surrounding inflammation are present. |

(Contd...)
### Table 5. (Continued).

| Time point | Group | Treatment | Animal number | Planar, site, and group histomorphology data | Micro-abscess | Anastomosis integrity | Tissue proliferation | Necrosis | Fibrosis/capsule formation | Comments/observations |
|------------|-------|-----------|---------------|---------------------------------------------|---------------|-----------------------|---------------------|----------|---------------------------|---------------------|
|            |       |           |               |                                             | 3             | 0                     | 0                   | 4        | 0                         | 2                   |
|            |       |           |               |                                             | 4             | 0                     | 0                   | 4        | 0                         | 2                   |
| Day 35: Group 1 - Vacuum (n=4) |       |           |               |                                             | 53184 Rectum ND | 0                     | NA                  | 0        | 0                         | None.               |
|            |       |           |               |                                             | Anastomosis Mean | 0.00                  | 0.00                | 4.00     | 0.00                      | 2.00                |
| Day 35: Group 2 - No Vacuum (n=2) |       |           |               |                                             | 52852 Rectum NP | 0                     | NA                  | 0        | 0                         | None.               |
|            |       |           |               |                                             | Anastomosis 1 | 0                     | 0                   | 4        | 0                         | 2                   |
|            |       |           |               |                                             | 2             | 0                     | 0                   | 4        | 0                         | 2                   |
|            |       |           |               |                                             | 3             | 0                     | 0                   | 4        | 0                         | 2                   |
|            |       |           |               |                                             | 4             | 0                     | 0                   | 4        | 0                         | 2                   |
|            |       |           |               |                                             | 52949 Rectum NP | 0                     | NA                  | 0        | 0                         | None.               |
|            |       |           |               |                                             | Anastomosis Mean | 0.00                  | 0.00                | 4.00     | 0.00                      | 2.00                |

Staples with surrounding inflammation are present. Presence of dilated cystic mucosal glands on the serosal surface with inflammation due to anastomosis procedure. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present. Staples with surrounding inflammation are present.

*Indication noted within mucosa with inflammatory infiltrate likely to be preexisting background finding or part of anastomosis site inadvertently captured in the section.

Day 35: Group 1 - Vacuum (n=4)

Day 35: Group 2 - No Vacuum (n=2)