

Pre-Clinical Review

Review of Bariatric and Colo-Rectal Animal Studies

Intraluminal Application of Peri-Strips Dry[®] with Veritas[®] Collagen Matrix Staple Line Reinforcement Material

*Synovis Surgical Innovations
Department of Research and Development*

ABSTRACT

Pre-clinical studies were conducted to assess the healing response and integrity over time of staple line anastomoses reinforced with linear and circular configurations of Peri-Strips Dry with Veritas Collagen Matrix (PSD Veritas) Staple Line Reinforcement. PSD Veritas was used to reinforce colon-colon, gastro-jejunum (G-J) and jejunum-jejunum (J-J) anastomoses. Stapled anastomoses reinforced with PSD Veritas and non-reinforced stapled anastomoses were evaluated for burst location, stricture, staple line integrity and histopathology at various time points up to 90 days. Anastomotic staple lines reinforced with PSD Veritas were more likely to burst at the adjacent intestine whereas non-reinforced anastomotic staple lines were more likely to burst at the staple line. PSD Veritas allows neo-vascularization and cellular ingrowth with conversion over time into histologically indistinguishable host tissue. There was no evidence of erosion, migration, stricture or infection.



Peri-Strips Dry with
Veritas Collagen Matrix
Linear and Circular
Staple Line Reinforcement
*(Circular Peel and Stick
version shown)*

BARIATRIC PORCINE STUDY

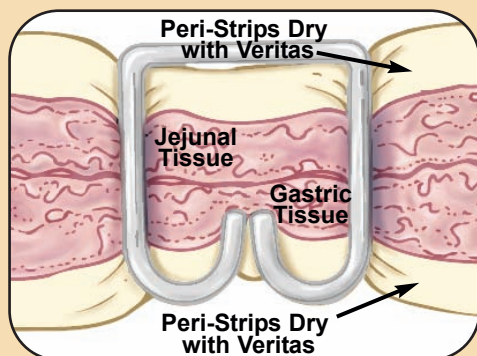
Overview

Staple line leak is a known complication of Roux-en-Y gastric bypass surgery. While there is a relatively low (2-6%) incident of staple line leaks, the outcome of a leak can be devastating¹. Clinical studies have shown that there is an initial decrease in burst strength of stapled anastomoses postoperatively and that the anastomoses are weakest at 3 days postoperative.^{2,3} Canine and porcine models have been used to assess the healing response of various stapled anastomoses.^{4,5,6,7} For this study a porcine model was chosen to assess the host healing response of non-reinforced and PSD Veritas reinforced J-J linear and G-J circular stapled anastomoses at 0, 3, 7, 14, and 42 days. The burst location of non-reinforced and PSD Veritas reinforced J-J linear stapled anastomoses was also evaluated at 0, 3, 7, and 14 days. All reinforced anastomotic staple lines were created by attaching the PSD Veritas to both the cartridge and anvil sides of the stapler.

Procedure

G-J Circular Stapled Anastomoses

One G-J circular anastomosis was created per pig in 23 pigs (total of 23 G-J circular anastomoses). The 0, 3, 7, and 42 day time points each included 3 PSD Veritas reinforced and 1 non-reinforced G-J circular anastomosis (4 pigs at each time point for a total of 16 pigs). The 14 day time point included 5 PSD Veritas reinforced and 2 non-reinforced G-J circular anastomoses (7 pigs total). The additional 3 pigs were added for reasons detailed in the ensuing discussion. G-J circular anastomoses were created with the 25mm Ethicon Endo-Surgery® Proximate® ILS Straight Intraluminal Stapler (Ethicon; Somerville, NJ). After locating the stomach through a small incision made in the abdominal wall, a gastrotomy was created for insertion of the stapler anvil. The anvil trocar was pushed through the stomach wall. A loop of small bowel was delivered into the field through a small window created in the mesentery. The bowel was dissected and the cartridge side of the circular stapler placed in the bowel. The inner shaft of the circular stapler was advanced to pierce through the bowel. The cartridge and anvil sides of the stapler were coupled, and the stapler fired creating a G-J circular stapled anastomosis. The gastrotomy was closed using standard suturing techniques. The pylorus remained open with this technique, making the G-J circular anastomoses a secondary opening for stomach evacuation. This procedure was selected to address the concern that a closed pylorus would be detrimental to the pigs and compromise long-term survival. To ensure accurate assessment of stricture, G-J circular anastomoses were created in 3 additional pigs included at the 14 day time point, 2 reinforced with PSD Veritas and 1 non-reinforced. In these three specimens, the pylorus was stapled shut using the Ethicon Endo-Surgery Endopath® ETS-45 linear cutting stapler (Ethicon, Somerville, NJ) with no staple line reinforcement, causing the G-J circular anastomosis to be the only evacuation opening. All pigs proceeded to the J-J linear



PSD Veritas reinforced anastomoses demonstrated no evidence of infection, acute inflammation, erosion or migration.

PSD Veritas reinforced J-J anastomoses are stronger than non-reinforced J-J anastomoses.

PSD Veritas allows cellular in growth and neo-vascularization as it is converted over time into histologically indistinguishable host tissue.

anastomotic procedure upon completion of the G-J circular anastomotic procedure except the 3 additional 14 day time point pigs which were utilized to assess G-J anastomotic stricture only.

J-J Linear Stapled Anastomoses

Two J-J linear anastomoses were created per pig in 20 pigs (total of 40 J-J linear anastomoses). All time points (0, 3, 7, 14 and 42 days) had 4 PSD Veritas reinforced and 4 non-reinforced J-J linear anastomoses (4 pigs at each time point). The J-J linear anastomoses were created with the Ethicon Endo-Surgery Endopath ETS-45 linear cutting stapler with green cartridges (4.1 mm) (Ethicon, Somerville, NJ). After locating the distal end of the dissected bowel from the G-J circular anastomosis procedure, another loop of small bowel (~18") was mobilized and an enterotomy created. The distal end of the first bowel section was anastomosed to the second section of bowel, creating a "y-shaped" J-J linear stapled anastomosis. Another loop of small bowel (~18") was mobilized and transected. The distal end of the first anastomosed segment was anastomosed to the proximal end of the second segment, alternating between PSD Veritas reinforced and non-reinforced anastomoses (2 per pig). The enterotomies were closed using standard suturing techniques. Metal ear tags with unique identification numbers were attached to the intestine at the anastomosis to aid in identification at explant. The bowel was observed for signs of ischemia and returned to the abdomen. The abdominal wall was closed in layers with 3-0 dexion interrupted suture being used for the peritoneum and internal rectus sheath, 2-0 ethibond interrupted suture for the rectus muscle and external rectus sheath, and a running 2-0 subcuticular suture for the skin.

Explant Protocol

At the appropriate time point, the pigs were euthanized intravenously with an overdose of KCl (One 3 day time point animal experienced an early non-buttrass related death, and therefore was not included in the study). All G-J circular and J-J linear anastomoses were evaluated for host healing response. The J-J linear anastomoses at 0, 3, 7, and 14 days were also evaluated for burst location.

Burst Testing

Each J-J linear anastomosis was identified by the ear tag. The bowel, at a point distal to the first J-J linear anastomosis but proximal to the second J-J linear anastomosis, was dissected. The proximal end of the intestine was closed off using hemostats or clamps. Tubing with connector was placed in the open end of the intestine, and the intestine securely attached to the connector with cable ties. Blue dye was pumped through the intestine and the staple line subjected to a constantly increasing rate of intraluminal pressure. Staple line leakage or bursting at the intestine was recorded at the first sign of leak or disruption. This procedure was repeated for the remaining J-J linear anastomoses.

No burst testing was performed on the G-J circular anastomoses (see Colo-Rectal Canine Study for burst testing of PSD Veritas reinforced and non-reinforced circular stapled colon-to-colon anastomoses).

Pathology Preparation

After the burst testing was completed, all G-J circular and J-J linear anastomoses were cleaned of extraneous materials. The staple lines were evaluated grossly and any unusual results noted. The stomal diameter of the G-J circular anastomoses was measured. The samples were placed in 10% formalin and prepared histologically at Pathology Associates, Division of Charles River Laboratories, Preclinical Services (Fredrick, MD).

Results

Burst

More non-reinforced J-J linear anastomoses burst at the staple line compared to PSD Veritas reinforced J-J linear anastomoses, 86% versus 47% respectively (see Figure 1). This difference in burst failure between PSD Veritas reinforced and non-reinforced anastomoses was statistically significant, $p=0.036$, z-test, Sigma Stat (see Table 1).

FIGURE 1

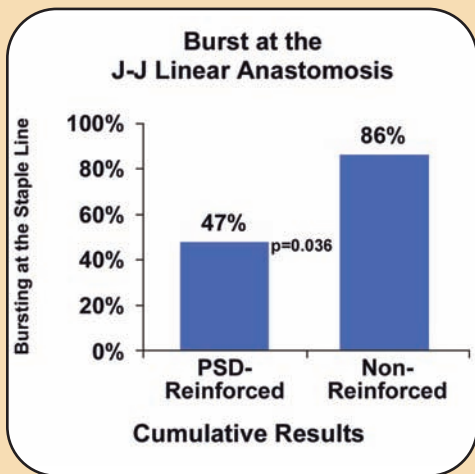


Table 1: Percent Bursting at the J-J Linear Anastomosis

Time Point	PSD Veritas Reinforced	Non-Reinforced	p-value
Day 0	25% (4)	100% (4)	*
3 Days	66% (3)	66% (3)	*
7 Days	66% (3)	100% (4)	*
14 Days	33% (3)	66% (3)	*
Cumulative	47% (13)	86% (14)	0.036

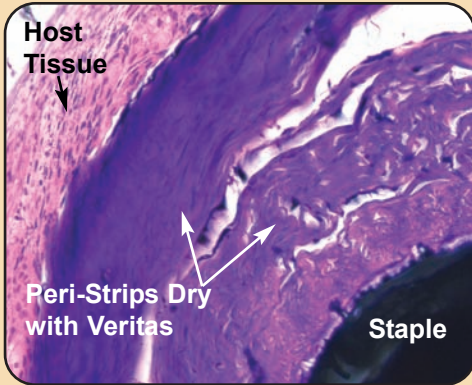
*Not applicable as sample size was too small to determine statistical significance (power <0.8).
() Sample size, some samples lost during explanting

Gross Pathology

There was no evidence at any time point of erosion or migration in any of the PSD Veritas reinforced or non-reinforced J-J linear or G-J circular anastomoses tested. The PSD Veritas staple line reinforcement material was readily apparent at 3 days, with some evidence of PSD-Veritas at 7 days in the J-J linear anastomoses. By 14 days, it was not possible to distinguish between a PSD Veritas reinforced and a non-reinforced J-J linear anastomosis.

In the animals with the sealed pylorus, there was no evidence of stricture in any of the PSD Veritas reinforced or non-reinforced G-J circular anastomoses (14 day time point). The diameter of the PSD Veritas reinforced and non-reinforced G-J anastomoses in the animals with the closed pylorus ranged from 9-10 mm. Because the G-J circular anastomosis was a secondary opening in the remaining

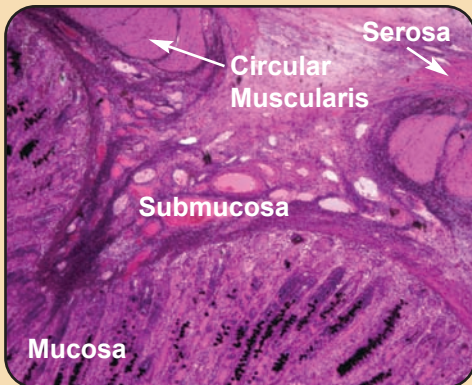
FIGURE 2: Histology Slides of G-J Circular Anastomoses



DAY 0



7 DAYS



42 DAYS

animals, closure over time occurred with both the PSD Veritas reinforced and non-reinforced G-J circular anastomoses.

Histopathology

There was no evidence of infection or acute inflammation with PSD Veritas reinforced G-J circular or J-J linear anastomoses. PSD Veritas supported cellular ingrowth by 3 days and neo-vascularization by 7 days. By 14 days there was no histological evidence of PSD Veritas at the implant site. PSD Veritas appeared to be replaced over time by histologically indistinguishable host tissue (see Figure 2).

Conclusions

- PSD Veritas reinforced J-J anastomoses are stronger than non-reinforced J-J anastomoses.
- PSD Veritas allows cellular ingrowth and neo-vascularization as it is progressively converted over time into histologically indistinguishable host tissue.
- PSD Veritas showed no evidence of infection, acute inflammation, erosion or migration.
- PSD Veritas reinforced G-J circular anastomoses demonstrated no difference in stricture as compared to the non-reinforced G-J circular anastomoses.

COLO-RECTAL CANINE STUDY

Overview

Anastomotic leak is a serious complication of colo-rectal surgery specifically during low anterior resections.⁸ Clinical studies have shown that postoperatively there is an initial decrease in burst strength in stapled anastomoses and that the anastomoses are weakest at three days postoperative.^{2,3} Canine and porcine models have been used to assess the healing response of various stapling devices.^{4,5,6,7} A canine model was chosen in this study.

This study evaluated the short and long-term healing and host tissue response of non-reinforced and PSD Veritas reinforced circular staple lines in the canine colon at 0, 3, 7, 14, 42 and 90 days. The burst location of non-reinforced and PSD Veritas reinforced anastomotic staple lines at 0, 3, 7, 14, 42 and 90 days were also evaluated. For evaluation at each time point, except 42 days, three (3) non-reinforced, and three (3) PSD Veritas reinforced staple lines were created using the USSC CEEA™ 28 mm circular stapler (Tyco Healthcare, USSC, Norwalk, Connecticut).

The animals at the 42 day time point had (4) four non-reinforced and (4) PSD Veritas staple lines. PSD Veritas was attached to both the cartridge and anvil sides of the stapler. Each animal received two anastomoses; one PSD Veritas reinforced and one non-

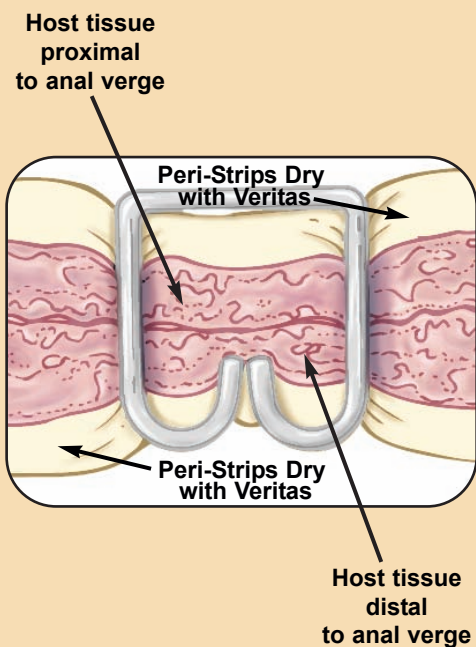
reinforced. Distance to the anal verge alternated between PSD Veritas reinforced and non-reinforced anastomoses.

Procedure

End-to-End Circular Anastomoses

The left colon was located via a median laparotomy along the linea alba. The colon was dissected >7 cm from the anal verge. The cartridge end of the circular stapler was inserted through the anus and the colon was attached using a purse string suture. The anvil side of the stapler was placed in the proximal end of the dissected colon and attached using a purse string suture technique. The cartridge and anvil sides of the stapler were coupled and the stapler fired, creating an end-to-end colonic anastomosis.

For the second anastomosis, the colon was dissected >5 cm from the transverse colon. The cartridge end of the circular stapler was inserted through the dissected colon which had been created to insert the anvil for the first anastomosis. The anvil of the circular stapler was inserted through an enterotomy created near the transverse colon. Both ends of the colon were attached to the stapler cartridge and anvil using a purse string suture technique. The cartridge and anvil sides of the stapler were coupled and the stapler fired, creating an end-to-end colonic anastomosis. The bowel was observed for signs of ischemia and returned to the abdomen. The enterotomy and abdominal incision were closed using standard surgical techniques.



Explant Protocol

At each follow-up time point, the dogs were euthanized intravenously with an overdose of sodium pentobarbital. The colon was located via a median laparotomy along the linea alba. The bowel, at points distal and proximal to each anastomoses, was dissected and removed for evaluation.

Burst Testing

The proximal end of the intestine was closed off using hemostats or clamps. Tubing with connector was placed in the distal end of the colon and the colon was securely attached to the connector with cable ties. Blue dye was pumped through the intestine subjecting the staple line to a constantly increasing rate of intraluminal pressure. At the first sign of leak or disruption it was recorded whether the failure was at the staple line or away from the staple line in the native intestine. This process was repeated for the other anastomosis in each animal.

Histopathology Preparation

After the burst testing was completed, the anastomoses were cleaned of extraneous materials. The staple lines were evaluated grossly and any unusual results noted. The samples were placed in 10% formalin and prepared histologically at River Valley Farms (Charles River Laboratories, Osceola, WI).

FIGURE 3:

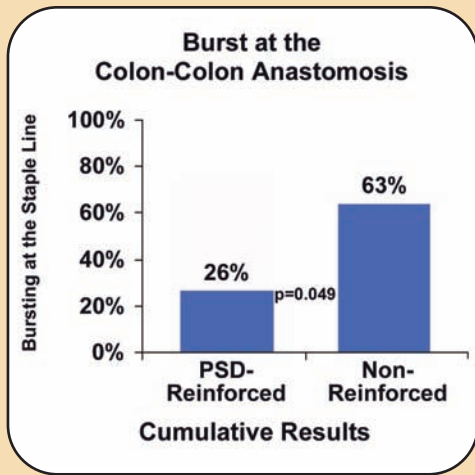
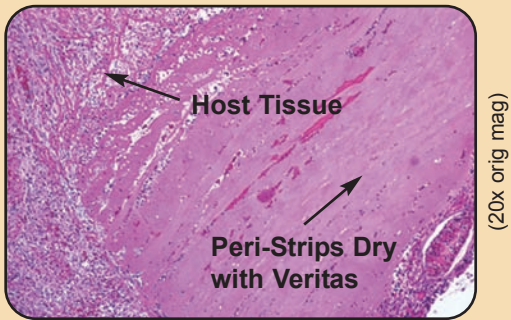
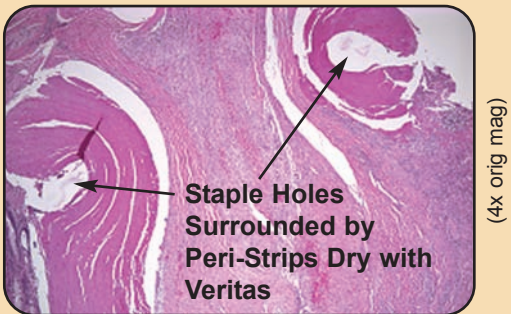


FIGURE 4: Histology Slides of Colon-Colon PSD Veritas Reinforced Anastomoses



7 DAYS



7 DAYS

Results

Burst

More non-reinforced anastomoses burst at the staple line compared to PSD Veritas reinforced anastomoses (see Table 3). 26% of the PSD Veritas reinforced anastomoses burst at the staple line compared to 63% of the non-reinforced anastomoses (see Figure 3). This difference in burst failure between PSD Veritas reinforced and non-reinforced anastomoses was statistically significant ($p = 0.049$, z-test, SigmaStat).

Table 3: Percent Bursting at the Colon-Colon Anastomosis

Time Point	PSD Veritas Reinforced	Non-Reinforced	p-value
Day 0	0% (3)	67% (3)	*
3 Days	100% (3)	100% (3)	*
7 Days	34% (3)	100% (3)	*
14 Days	0% (3)	67% (3)	*
42 Days	25% (4)	50% (4)	*
90 Days	0% (3)	0% (3)	*
Cumulative	26% (19)	63% (19)	0.049

*Not applicable as sample size was too small to determine statistical significance (power <0.8).
() Sample size

Gross Pathology

There was no evidence of erosion, migration or stricture at any time point. The PSD Veritas staple line reinforcement material was readily apparent at 3 days, with some evidence at 7 days (see Figure 4). By 14 days, it was not possible to distinguish between a PSD Veritas reinforced anastomosis and a non-reinforced anastomosis.

Histopathology

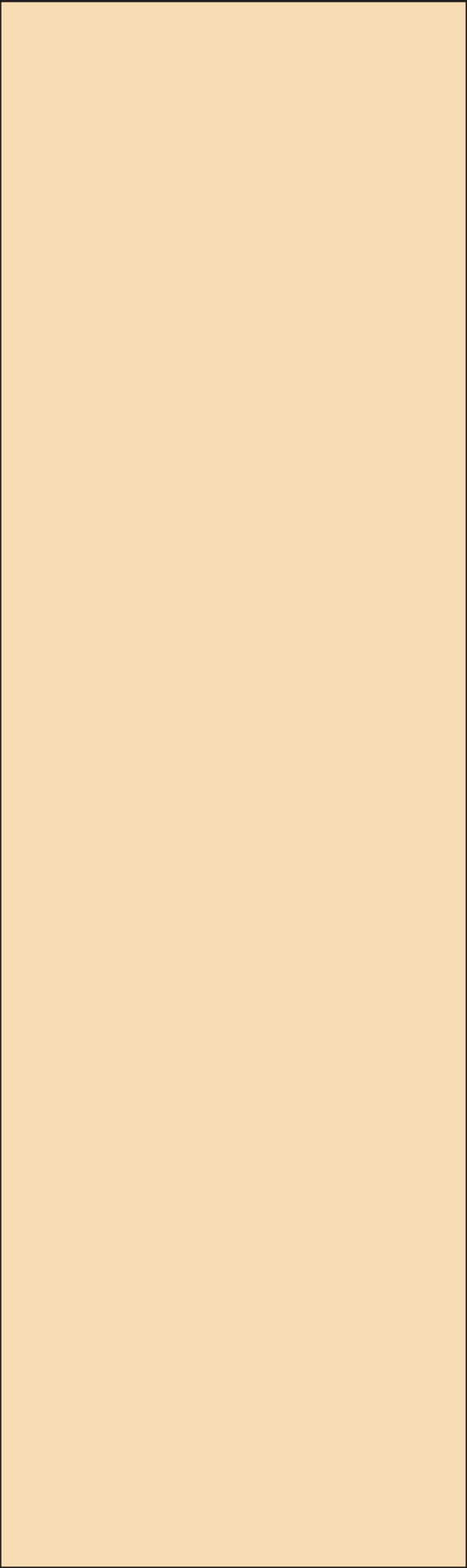
There was no evidence of infection or acute inflammation with PSD Veritas. PSD Veritas allowed cellular ingrowth by 3 days and neo-vascularization by 7 days. By 14 days there was no histological evidence of PSD Veritas at the implant site. PSD Veritas was apparently replaced over time by histologically indistinguishable host tissue.

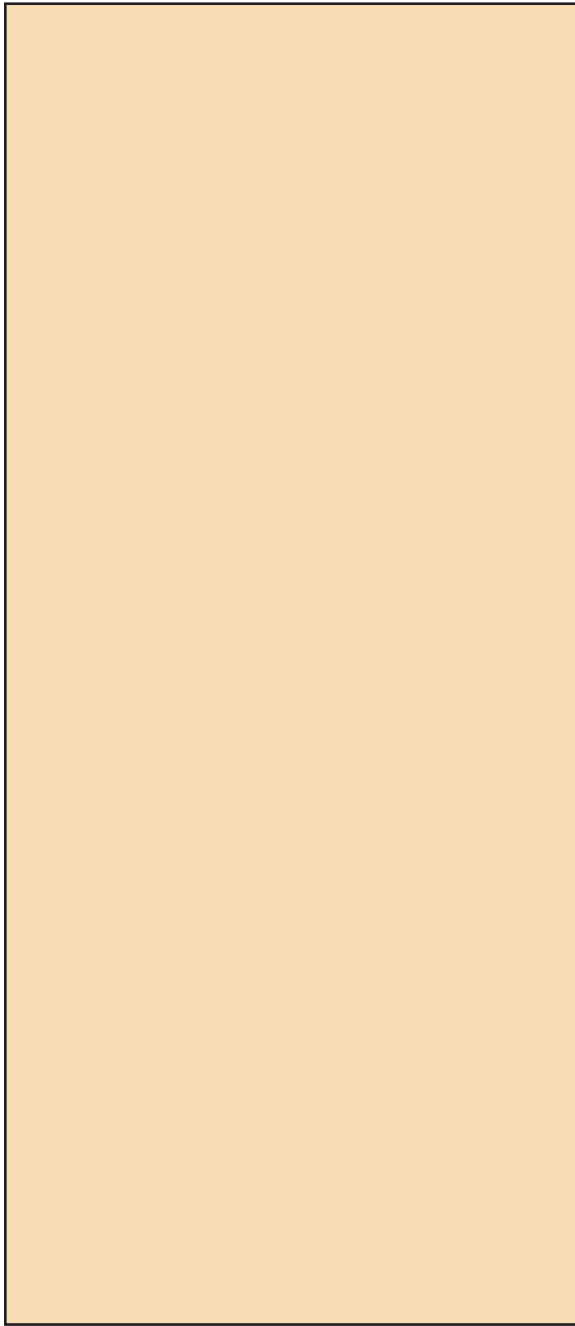
Conclusions

- Significantly less PSD Veritas reinforced anastomoses burst at the staple line as compared to non-reinforced anastomoses.
- PSD Veritas allows cellular ingrowth and neo-vascularization as it is progressively converted over time into histologically indistinguishable host tissue.
- PSD Veritas showed no evidence of infection, acute inflammation, erosion or migration.
- PSD Veritas reinforced colon-to-colon circular anastomoses demonstrated no difference in stricture as compared to the non-reinforced colon-to-colon circular anastomoses.

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2575 University Ave. W.
St. Paul, MN 55114-1024 USA
651.796.7300 800.255.4018
651.642.9018 (fax)
synovissurgical.com

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