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Technical Report

Evaluation of Unmeshed and 1:1 Meshed AlloDerm Bolsters for Stapled Rectal Anastomoses in a Porcine Model

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Abstract

Introduction: The major morbidity of colorectal anastomoses is leaks. The concept of staple-line reinforcement is a growing area of interest. In this study, we evaluated the feasibility and effect of utilizing AlloDerm to bolster end-to-end stapled rectal anastomoses in a porcine model.
Methods: A total of 30 female 45-kg domestic pigs were studied, and each served as its own control by creating a bolstered and unbolstered anastomosis in each animal. All anastomoses were created with a 29-mm end-to-end stapling device. Bolstered anastomoses were randomized to proximal and distal positions along the rectum, and each rectorectal anastomosis was separated by an average of 10 cm. In 20 pigs, an unmeshed bolster of a 0.5–0.7-mm thickness was used. The remaining 10 pigs had a 1:1 meshed bolster that was 0.34–0.51 mm thick. The animals were survived for 14 days. Barium enemas were then performed and the two anastomotic sites harvested, and each anastomosis underwent burst testing. The internal diameter of each anastomosis was measured and a biochemical analysis was performed for matrix metalloproteinase (MMP), elastin and collagen content.
Results: The unmeshed bolstered anastomoses burst fewer times than the unbolstered anastomoses ($P = 0.004$) and had higher burst pressures ($P = 0.023$), though their anastomotic circumferences were smaller ($P = 0.007$). Meshed bolsters offered no strength advantage to anastomoses and were significantly ($P = 0.009$) smaller than unbolstered anastomoses in the same animal. No difference in elastin, collagen, or MMP content was observed between bolstered and unbolstered groups. No animals had clinical or radiographic leaks.
Conclusions: The routine use of unmeshed and 1:1 meshed AlloDerm bolsters is safe and does not appear to inhibit healing in elective colorectal surgery on healthy subjects. AlloDerm may have a role as a tissue bolster in select patients who are more prone to develop anastomotic leaks.

Introduction

The most feared morbidity after a colorectal resection is an anastomotic leak. The average clinical leak rate for stapled colorectal anastomoses is approximately 4–5% and can increase to 15–20% for low colorectal and coloanal anastomoses.[1–3] The range of clinical leak rates after low anterior resection of the rectum is 3–39%,[4–8] and radiographic leak rates have an even higher incidence, depending on their timing after surgery.[9] Patients are usually quite ill after an anastomotic leak, and the estimated mortality from this complication can be greater than 20%.[10] Anastomotic leaks are the primary cost driver in patients undergoing a low anterior resection,[11] and long-term oncologic outcomes and recurrence

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rates are worse[12,13] in patients who experience this complication.

The concept of using biologic materials as a bolster to reinforce a stapled anastomosis has received limited attention in the past but is an idea that has experienced a recent resurgence in interest. There are several products currently available for staple line reinforcement. These include bovine pericardium, bovine collagen strips, nonabsorbable expanded polytetrahydroethylene, and absorbable polymer films. Most of these products are placed on the staple line prior to firing the device, with the bolster acting both as a scaffolding to promote healing and as a tissue barrier to prevent anastomotic leakage. Little has been accomplished to better establish what the exact role of these products is and in which patients their use is most beneficial and cost-effective.

AlloDerm (LifeCell Corp., Branchburg, NJ) is an acellular dermal matrix that is developed from human cadaver skin through a process where the cadaveric epidermis and dermis are separated by using a high-ionic-strength solution. Any dermal cells are washed away by using sodium deoxycholate as part of a proprietary processing, and the tissue is freeze-dried without forming ice crystals, which would damage the tissue. The final product contains human dermal collagen, elastin, and dermal matrix and acts as scaffolding for healing by promoting tissue ingrowth. The processing of this tissue is somewhat unique among other biologic materials, because the method by which the tissue is washed to obtain acellularity does not prevent the final product from promoting a migration of the recipient body's cells and, subsequently, complete incorporation into the body. Rather than acting as a permanent foreign material that is encapsulated, AlloDerm allows angiogenesis and cellular ingrowth and literally becomes a part of the patient.

In this study, we investigated the use of unmeshed and 1:1 meshed versions of AlloDerm as staple-line bolsters for rectal anastomoses in order to determine whether their reinforcement of the anastomotic staple line would provide additional strength. Additionally, the affect of each bolster on anastomotic circumference was studied.

Methods

This was an investigator-initiated study, with a grant from LifeCell, Inc., and was performed with approval from our institution's Animals Studies Committee and Department of Comparative Medicine. A total of 30 female 45-kg domestic pigs were studied, and each served as its own control by creating a bolstered and unbolstered anastomosis in each animal. All anastomoses were created with a 29-mm end-to-end anastomotic (EEA) stapling device. Bolstered anastomoses were randomized to proximal and distal positions along the rectum, and each rectorectal anastomosis was separated by an average of 10 cm. In 20 pigs, an unmeshed bolster of a 0.5–0.7-mm thickness was used and the preliminary analysis showed a noncompliant anastomosis in the bolstered group. The remaining 10 pigs had a 1:1 meshed bolster that was 0.34–0.51 mm thick. In all cases, the AlloDerm required reconstitution with sterile saline for several minutes prior to use.

Upon arrival to our facility, each pig was allowed 72 hours to acclimate before any preparations for surgery were made. Starting 48 hours prior to surgery, each pig was given a mechanical bowel preparation with 1 gallon of NuLYTELY each day, with the addition of electrolyte-rich liquid three times a day in order to provide adequate hydration and calories. On the day of surgery, each pig was only allowed water. Each animal received antibiotic prophylaxis with a 20-mg/kg injection of intramuscular (IM) cefazolin that was started preoperatively less than 1 hour prior to the skin incision and was continued orally every 12 hours until the end of postoperative day 4. All surgeries were open and performed under general anesthesia with the supervision of a licensed veterinary technician (KC) in a Food and Drug Administration (FDA)-approved animal facility at the Washington University Institute of Minimally Invasive Surgery (WUIMIS; St. Louis, MO). All surgeries were performed through a 6-cm low midline incision and in the following manner. The proximal anastomosis was formed first, using a linear cutting 75-mm blue stapler to transect the rectum. The proximal staple line was excised, and a full-thickness purse-string suture was then placed in the proximal rectum by using a 2-0 monofilament suture to secure the anvil. An assistant then introduced the base of the device into the rectum and deployed the pin through the center of the rectal stump. The anvil and pin were joined, taking care to avoid any twisting of the rectum. For anastomoses that were bolstered, the AlloDerm was placed onto the distal rectal segment by pushing it onto the pin of the stapler after passage through the rectal stump. Once the two segments of bowel were fully apposed, a final inspection was made to ensure that no extraneous tissue had been pulled into the device. The device was then fired and the base was removed. Excess AlloDerm was trimmed from the circumference of each bolstered staple line. Both donuts were checked to ensure full-thickness tissue presence and concentricity. The anastomosis was not leak tested. The distal anastomosis was then created 10 cm along the rectum with exactly the same steps, so that each animal had one bolstered and one unbolstered anastomosis. No irrigation of the abdomen or wound was performed. The fascia was reapproximated by using 0-Prolene (Ethicon, Cincinnati, OH), placed with an interrupted figure-of-eight technique. The subcutaneous tissue was subsequently closed by using a running 2-0 Vicryl (Ethicon) suture. The incision was anesthetized by using 20 mL of 1% Marcaine® (Abbott Laboratories, Abbott Park, IL) placed subcutaneously, and the skin edges were approximated by using skin staples. A viscous skin adhesive was also used to cover the skin incision. At this point, the surgery was terminated and the pig was taken to a recovery room and extubated.

Each pig was monitored for the first 24 hours in a recovery room, which served as a step-down unit. The pigs were given IM buprenorphine (0.02 mg/kg) for analgesia during the evening of surgery and were also orally administered 200 mg of the nonsteroidal anti-inflammatory (NSAID), carprofen (Pfizer, New York, NY) during the first 24 hours. The pigs were started on a clear liquid diet for the first 48 hours after surgery, followed by balanced calorie, protein, carbohydrate, and fat shakes for the next 24 hours. Mash was begun the following day (postoperative day 3), and by postoperative day 4, the pigs were on pig chow. On postoperative day 14, the pigs were sedated with an IM cocktail of 2 mL of telazol (Wyeth, Madison, NJ), 250 mg of ketamine, and 250 mg of xylazine (Bayer, Levekussen, Germany) and then given pentobarbital for euthanasia. Postnecropsy evaluation of the pig rectums differed between the unmeshed and meshed bolster groups.

Unmeshed bolster group

At necropsy, the rectum was removed from the pig. The rectum was then divided so that at least 5 cm of native bowel was present on either side of each anastomosis. Each anastomosis was separately burst tested to accurately determine anastomotic strength. One end of the bowel was closed with a noncrushing bowel clamp while a plastic tube was secured in the other end, using a plastic zip tie. The bowel was completely submersed in a large bowl filled with saline. A mercury hand-operated sphygmomanometer was used to steadily inflate the bowel with air. The pressure at which air bubbles were first noted from the anastomosis was recorded as the burst pressure. If native tissue (i.e., a segment of rectum uninvolved in the anastomosis) burst and the anastomosis was intact, this was not considered a burst pressure, and no pressure was recorded if the burst test could not be repeated due to the location of the tear in the native tissue with respect to the anastomosis. Each of the anastomoses was then excised circumferentially and the circumference of each was measured.

Meshed bolster group

Each pig rectum was excised at necropsy and a barium radiograph was obtained to evaluate the diameter of each anastomosis, compared to the surrounding native bowel. The rectum was then divided so that at least 5 cm of native bowel was present on either side of each anastomosis. Each anastomosis was then tested to determine failure pressures separately to ensure accuracy of the measurements. One end of the bowel was closed with a noncrushing bowel clamp while a plastic tube was placed in the other end of the bowel segment and secured by using a plastic zip tie. The bowel was completely submersed in a large container filled with room-temperature saline. A hand-operated mercury sphygmomanometer was used to steadily inflate the bowel with air. One examiner was assigned to observe the saline bath for the presence of any air bubbles, while a second examiner monitored the pressure reading on the sphygmomanometer to record the pressure at the exact moment of failure. The pressure recorded was either the first pressure where air bubbles were observed, regardless of whether the anastomosis or native tissue had failed; or, in cases when the anastomosis did not leak, the pressure recorded was the maximal pressure reached. The presence or absence of failure for each anastomosis was recorded.

Following this, each anastomosis was excised circumferentially and four sections of tissue immediately adjacent to each anastomosis were harvested for tissue analysis. Samples from each anastomosis were subsequently studied with gel zymography to estimate the concentration of MMP-2 (matrix metalloproteinase) and -9. Tissue was prepared by pulverization under liquid nitrogen, followed by extraction in ice-cold Tris-HCL buffer, with a pH of 7.5. This extraction solution also contained 1.0 mol/L of NaCL, 2.0 mol/L of urea, 0.1% (w/v) of Brij-35, 0.1% ethylenediamine tetraacetate, and a mixture of protease inhibitors. After centrifugation at 10,000g for 1 hour at 4°C, the supernatant was centrifugally concentrated by using a 5000-molecular-weight cut-off membrane for 2 hours at 4°C. Samples consisting of 40 μg of total protein per gel lane were then subjected to gelatin zymography under nondenaturing conditions and electrophoretically resolved through 10% polyacrylamide gels that were copolymerized with 1 mg/mL of gelatin substrate. The gels were then stained by using 0.5% Coomassie Blue R-250 in 40% methanol/10% acetic acid (Sigma Chemical, St. Louis, MO), and gelatin-degrading activities were observed as clear bands against a dark background of intact substrate. The relative molecular-weight of each proteolytic band was determined by the migration positions of known molecular-weight standards (Bio-Rad, Richmond, CA) and authentic 92- and 72-kDa gelatinase standards. The gels were dried, scanned, and the relative amount of each gelatinase activity was estimated by densitometry.

Radioimmunoassay (RIA) techniques were used to quantify desmosine concentrations, as a marker for elastin, and an amino-acid analysis was performed to quantify the concentration of hydroxyproline, as a marker for collagen from tissue samples taken immediately adjacent to each anastomosis. The internal diameter of each skeletonized anastomosis was then measured and the tissue was preserved in 10% formalin for paraffin sectioning and staining for collagen and elastin, using trichrome and Verhoef-Van Gieson (VVG) stains, respectively.

Statistical methods

The animals with unmeshed bolsters were compared within the group and the animals with meshed bolsters were compared within the group, and then the meshed and unmeshed groups were compared for final analysis. The mean values of burst and failure pressures, anastomotic circumferences, and MMP-2 and -9, elastin, and collagen content were compared for significant differences by using a Student's t-test. The absolute difference between the initial and 2-week anastomotic circumferences was calculated for each type of anastomosis, and a t-test was used to compare the degree of dilation between bolstered and unbolstered anastomoses. A Fisher's exact test was used to compare the presence of adhesions to the anastomoses in the animals with meshed bolsters.

Results for the unmeshed AlloDerm bolster ($n = 20$)

The results for the 2-week failure burst pressures in pigs with unmeshed bolsters are listed in Table 1. A total of 4 of 20 bolstered anastomoses burst, compared to 13 of 20 unbolstered anastomoses (Fisher's exact test; $P = 0.004$). The mean burst pressure of the unmeshed bolstered anastomoses was 263 $\pm$ 45 mm Hg (median, 258; range, 215–320) and was 198 $\pm$ 44.9 mm Hg for the unbolstered anastomoses (median, 195; range, 122–277), a difference that was significant (Student's t-test; $P = 0.023$). Comparison of distally placed anastomoses as a separate group revealed no difference in median burst pressures (bolstered, 240; unbolstered, 240; $P = 0.121$). Proximally placed anastomoses could not be compared as a group due to only one proximal bolstered anastomosis having burst.

The mean internal circumference (Table 2) of the unmeshed bolstered anastomoses measured at tissue harvest at 14 days was 9.98 $\pm$ 1.71 cm, while the mean internal circumference for the unbolstered anastomoses was 11.4 $\pm$ 1.47

Table 1. Anastomotic Failure Pressures in Unmeshed Bolster Group at 2 Weeks

Position of bolster in animal	*Burst pressure of bolstered anastomoses (mm Hg)*	*Burst pressure of unbolstered anastomoses (mm Hg)*	P-*value from Student's* t-*test*
Proximal	Mean: N/A	Mean: 214 ± 20.2	N/A
#1	*	245	
#2	*	*	
#3	*	230	
#4	*	200	
#5	*	192	
#6	*	*	
#7	*	*	
#8	*	*	
#9	320	210	
Distal	244 ± 30.7	187 ± 53.2	0.121
#1	*	245	
#2	*	*	
#3	*	122	
#4	*	190	
#5	*	195	
#6	276	277	
#7	*	145	
#8	*	*	
#9	*	*	
#10	240	180	
#11	215	140	
Overall	263 ± 45.6	198 ± 44.9	0.023

An asterisk "*" denotes that the anastomosis did not burst.

Table 2. Anastomotic Circumferences of Unmeshed Bolster Group at 2 Weeks

Position of bolster	*Bolstered anastomotic circumference (cm)*	*Unbolstered anastomotic circumference (cm)*	P-*value from Student's* t-*test*
Proximal			0.027
#1	6.2	9.4	
#2	8.4	9.5	
#3	8.8	10.0	
#4	8.9	10.5	
#5	9.1	11.1	
#6	9.6	11.1	
#7	9.8	12.2	
#8	11.2	13.5	
#9	12.0	13.9	
	Mean: 9.38 ± 1.64	Mean: 11.3 ± 1.64	
Distal			0.121
#1	8.3	9.5	
#2	8.7	10.1	
#3	9.0	10.4	
#4	9.2	10.8	
#5	9.4	10.9	
#6	10.7	11.4	
#7	11.0	11.9	
#8	11.0	12.0	
#9	11.7	12.3	
#10	11.8	13.5	
#11	14.0	14.0	
	Mean: 10.5 ± 1.68	Mean: 11.5 ± 1.39	
Overall	9.98 ± 1.71	11.4 ± 1.47	0.007

Table 3. Anastomotic Failure Pressures in Meshed Bolster Group at 2 Weeks

Position of bolster in animal	*Failure pressure of bolstered anastomoses (mm Hg)*	*Failure pressure of unbolstered anastomoses (mm Hg)*	P-*value from Student's t-test*
Proximal			0.180
#1	258	263	
#2	280	180	
#3	300	260	
#4	238	168	
#5	Unable to test	268	
	Mean: 269 ± 26.9	Mean: 228 ± 49.4	
Distal			0.339
#1	300	170	
#2	268	300	
#3	300	250	
#4	268	260	
#5	232	Unable to test	
	Mean: 274 ± 28.2	Mean: 245 ± 54.5	
Overall	272 ± 26	235 ± 49.1	0.069

cm, a significant difference ($P = 0.007$). Comparing mean proximal anastomotic circumferences revealed a significant difference between the two types of anastomoses (bolstered, 9.38 ± 1.64 cm; unbolstered, 11.3 ± 1.64 cm; $P = 0.027$), while a comparison of distal anastomoses did not show a significant difference (bolstered, 10.5 ± 1.68 cm; unbolstered, 11.5 ± 1.39 cm; $P = 0.121$). The initial internal anastomotic circumference for all anastomoses was 5.96 cm, since a 29-mm EEA stapling device was used in each case. The mean increase in circumference over the 2 weeks was, therefore, 67% for the bolstered anastomoses and 91% for the stapled anastomoses. The absolute difference between the 2-week and initial circumferences for each type of anastomosis in the unmeshed bolster group was compared by using a Student's *t*-test. The degree of dilation for all unmeshed bolstered, compared to unbolstered, anastomoses was significant ($P = 0.008$). The proximally positioned unbolstered anastomoses were significantly larger than the bolstered anastomoses ($P = 0.027$). Comparing distal anastomoses did not reveal a significant difference in degree of dilation ($P = 0.124$).

Results for the 1:1 meshed AlloDerm bolster (n = 10)

None of the meshed bolstered or unbolstered anastomoses failed during testing. The tissue failure pressures were similar at 2 weeks after surgery (Table 3). The mean tissue failure pressure was 272 ± 26 mm Hg (median, 268; range, 232–300) for the bolstered anastomoses and 235 ± 49.1 mm Hg (median, 260; range, 168–300) for the unbolstered anastomoses ($P = 0.069$). Comparison of proximally and distally placed anastomoses as separate groups revealed no differ-

Table 4. Anastomotic Failure Pressures in Unmeshed Bolster Group at 2 Weeks

Position of bolster	*Bolstered anastomotic circumference (cm)*	*Unbolstered anastomotic circumference (cm)*	P-*value from Student's* t-*test*
Proximal			0.425
#1	10.4	10.4	
#2	10.0	10.8	
#3	11.6	10.0	
#4	10.0	10.4	
#5	10.0	13.0	
	Mean: 10.4 ± 0.693	Mean: 10.9 ± 1.2	
Distal			0.006
#1	9.0	12.0	
#2	10.0	12.0	
#3	9.6	12.2	
#4	10.0	12.0	
#5	11.6	11.0	
	Mean: 10 ± 0.963	Mean: 11.8 ± 0.477	
Overall	10.2 ± 0.813	11.4 ± 0.986	0.009

ence in median failure pressures, based on the position along the rectum (Table 3). The last pig to be sacrificed had an unusually short rectum, and despite attempts to separate the anastomoses by 10 cm to allow for the testing of failure pressures, this could not be done.

The mean internal circumference (Table 4) was 10.2 ± 0.813 cm for the bolstered anastomosis and 11.4 ± 0.986 cm for the unbolstered anastomosis ($P = 0.009$). Comparing mean proximal anastomotic circumferences revealed no difference in circumference between the two types of anastomoses (Table 4), while unbolstered distal anastomoses were significantly larger ($P = 0.006$). The bolstered anastomosis actually increased in size from the initial 5.96 cm by 71%, while the unbolstered anastomosis grew in circumference by 91%. The absolute difference between the 2-week and initial circumferences for each type of anastomosis was compared by using a Student's *t*-test. There was a significant difference in the degree of dilation between all bolstered and all unbolstered anastomoses ($P = 0.009$) and when the anastomoses were compared according to distal position ($P = 0.006$). However, proximal anastomoses did not expand differently ($P = 0.425$).

Biochemical analysis of the meshed group

The portions of intestine harvested from the anastomotic line of the pigs in the meshed group were processed for gel zymography to determine the amount of MMP-2 and -9 at the anastomosis (Fig. 1). There was no difference in MMP-2 and -9 concentrations between bolstered and unbolstered anastomoses, and the position of the bolster (i.e., proximal or distal) did not affect this finding. There was almost exactly the same band position on the gel for both bolstered and unbolstered anastomoses, indicating almost identical concentrations of MMP for each technique.

The elastin content of each anastomosis was analyzed by measuring the concentrations of the amino acid, desmosine, in picomoles of desmosine (D) per milligram of protein (P), using an RIA technique. There was no difference ($P = 0.49$) in the elastin content between bolstered and unbolstered anastomoses (bolstered: mean, 103 ± 31.2 pmD/mgP; unbolstered; mean, 115 ± 46.1 pmD/mgP). Separating the anastomoses by their position in the rectum also showed no difference in elastin concentrations (Table 5).

Hydroxyproline concentrations were measured by using amino-acid analysis in order to quantify the collagen content of each anastomosis. As with elastin, there was no difference between hydroxyproline concentrations (Table 6) in either anastomosis ($P = 0.48$) (bolstered: mean, 226 ± 52.9 pmD/mgP; unbolstered: mean, 264 ± 159 pmD/mgP) or in either position (Table 6).

The results of Masson's trichrome (collagen) and VVG (elastin) staining revealed similar staining patterns, consistent with similar collagen and elastin concentrations in bolstered and unbolstered anastomoses. The AlloDerm bolster could be seen at the anastomotic line incorporated and infiltrated with fibroblasts without encapsulation.

Barium radiographs (Fig. 2) were obtained on each rectum after their removal at autopsy. There were no radiographic leaks in any of the specimens. Comparing the width of the barium column across the unbolstered and bolstered anastomoses in each rectum demonstrated a consistent 1.3:1 (unbolstered:bolstered) ratio in the width of the anastomotic shadow.

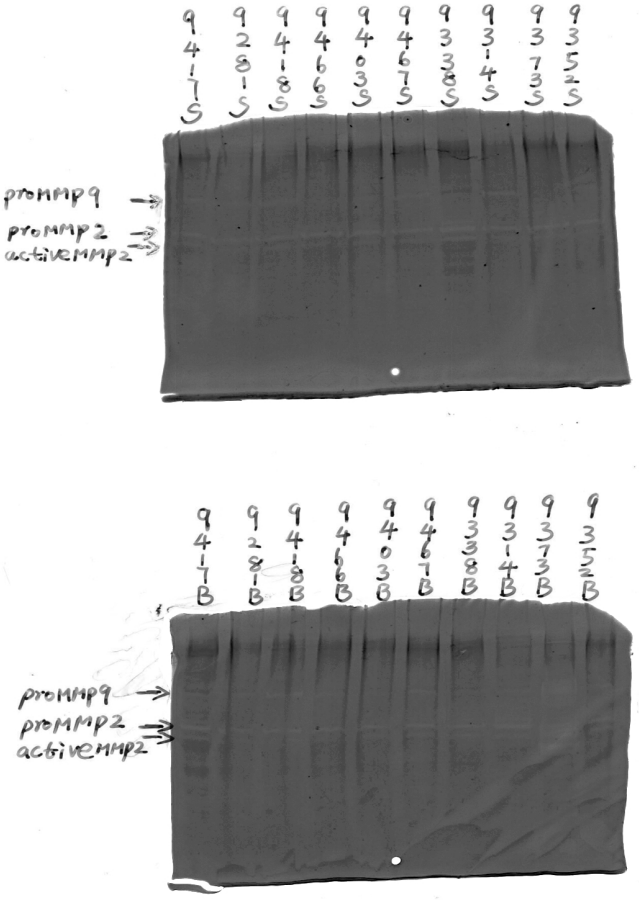

FIG. 1. Gel zymography for matrix metalloproteinase-2 and -9 content in meshed bolstered and unbolstered anastomoses.

Dense adhesions requiring careful, sharp adhesiolysis were found in 8 of the 10 bolstered anastomoses, and 6 of the 10 unbolstered anastomoses had similar adhesions (Fisher's exact test; $P = 0.628$). There were no postoperative complications in the study. None of the animals experienced an anastomotic leak, either clinically or on barium radiography. There was no difference in the ability of animals to tolerate an advancement of their diet or in their bowel habits, and all animals had their diets advanced equally. There was no difference in their requirement for analgesics. Compared to the unbolstered anastomoses, an average of 7 minutes of additional time was required to position the AlloDerm on the stapling device and trim excess AlloDerm from the anastomosis after firing and removing the stapler.

Comparison of unmeshed versus meshed bolsters

A total of 4 of 20 unmeshed bolstered anastomoses burst, compared to 0 of 9 (1 anastomoses could not be tested) of the meshed bolstered anastomoses ($P = 0.204$). There was no difference ($P = 0.667$) comparing the mean 2-week burst pressures for the unmeshed bolsters (263 ± 45 mm Hg) to the mean 2-week failure pressures for the meshed bolsters (272 ± 26 mm Hg). A comparison of proximally placed bol-

Table 5. Desmosine Concentrations of Anastomoses in Unmeshed Bolster Group at 2 Weeks

Position of bolster	*Desmosine in bolstered anastomoses (picomoles/mg protein)*	*Desmosine in unbolstered anastomoses (picomoles/mg protein)*	P-*value from Student's* t-*test*
Proximal			0.567
#1	135	98	
#2	91	101	
#3	65	120	
#4	83	88	
#5	159	68	
	Mean: 107 ± 39	Mean: 95 ± 19	
Distal			0.236
#1	92	147	
#2	81	205	
#3	143	100	
#4	92	168	
#5	86	56	
	Mean: 98.8 ± 25.1	Mean: 135 ± 58.3	
Overall	Mean: 103 ± 31.2	115 ± 46.1	0.490

sters could not be performed, since only 1 proximal unmeshed bolster burst; distally placed bolsters showed no difference ($P = 0.208$).

There was no difference ($P = 0.675$) between the mean 2-week circumferences for unmeshed bolsters (9.98 ± 1.71 cm) and meshed bolsters (10.2 ± 0.81 cm). Comparison of proximally ($P = 0.214$) and distally ($P = 0.609$) placed anastomoses as separate groups did not demonstrate a difference. There was no difference in the degree of anastomotic dilation between all unmeshed and all meshed bolsters ($P = 0.675$), including a comparison of proximally ($P = 0.214$) and distally ($P = 0.609$) placed bolsters as groups. There were no radiographic leaks or clinical leaks in either the unmeshed or meshed groups.

Discussion

The "perfect" anastomosis, one which does not leak or stricture, has not been created. The development of stapling devices has decreased the technical difficulty of colorectal surgery and the time required[14–16] for the construction of low colorectal anastomoses. These devices allow an anastomosis to be created consistently where one could not be constructed[17] with suturing techniques. In elective surgery, stapling devices have been shown to be just as safe as hand sewing.[18–21] However, leak rates with stapled anastomoses have not been shown to be lower, when compared to hand-sewn anastomoses.[22] Stricture rates vary widely for all of the various anastomotic techniques. The definition of a stricture

Table 6. Anastomotic Failure Pressures in Unmeshed Bolster Group at 2 Weeks

Position of bolster	*Hydroxyproline in bolstered anastomoses (nanomoles/mg protein)*	*Hydroxyproline in unbolstered anastomoses (nanomoles/mg protein)*	P-*value from Student's* t-*test*
Proximal			0.56
#1	329	114	
#2	210	192	
#3	202	255	
#4	197	197	
#5	142	206	
	Mean: 216 ± 68.6	Mean: 193 ± 50.7	
Distal			0.165
#1	193	191	
#2	276	450	
#3	269	630	
#4	207	134	
#5	234	271	
	Mean: 236 ± 36.7	Mean: 335 ± 203	
Overall	226 ± 52.9	264 ± 159	0.480

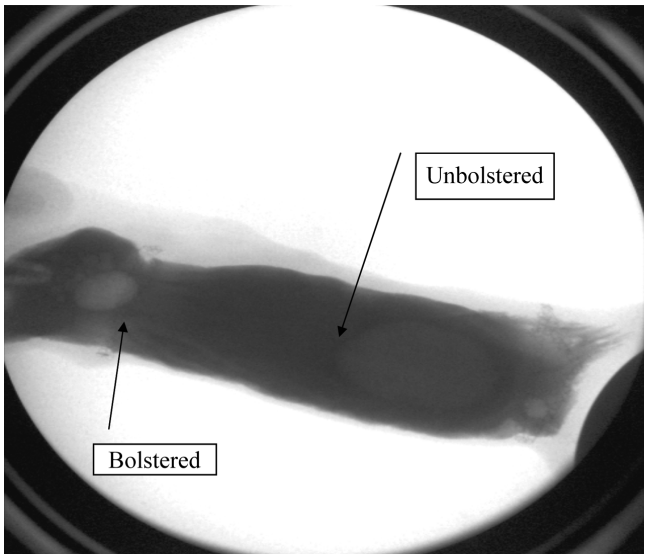

FIG. 2. Barium radiographs of meshed bolstered and unbolstered anastomoses in continuity.

also varies depending on the requirement of symptoms, imaging for measurement of luminal circumference, and length of follow-up. The rate of stricture formation does not appear to be consistently lessened with the use of stapling devices.[23] The application of biologic materials to the stapled anastomosis may reduce the leak rate from stapled anastomoses but needs to be studied before offering this approach to all patients.

The unmeshed bolstered anastomoses burst less frequently than the unbolstered controls, while the meshed bolster did not offer a strength advantage. It is possible that the meshing process weakened the bolster sufficiently to remove any improvement in burst strength (the lack of a difference in the presence of adhesions between meshed bolstered and unbolstered anastomoses suggests this, as discussed below). Another possible explanation why the meshed bolsters did not produce stronger burst strengths may relate to the technique of burst testing between the meshed and unmeshed bolster groups. In the pigs with a meshed bolster, an extensive and meticulous sharp adhesiolysis was performed to completely skeletonize the bowel wall around the anastomosis prior to burst testing, taking extreme care not to cut the bowel wall during this process. However, in the animals with unmeshed bolsters, the adhesions were left in place. Whether the presence of the adhesions offered additional resistance to bursting or whether adhesiolysis weakened the bowel wall are both possibilities.

Anastomotic strength at 14 days was not influenced by the presence of a meshed AlloDerm bolster in this study. None of the anastomoses in the meshed bolster group failed during burst testing; in cases where tissue did burst, it always involved native tissue and not the anastomosis. The median maximal test pressures for both meshed bolstered and unbolstered anastomoses was over 200 mm Hg, pressures which are much higher than would be experienced in any clinical setting. This indicates that both types of anastomoses provide adequate strength after a period of postoperative healing after elective surgery in healthy subjects. Whether AlloDerm would help to improve the strength of an anastomosis at a time after surgery prior to 2 weeks, when created under emergent conditions, or in the nonideal setting of chronic steroid use, irradiated tissue, active infection, or clinical shock, is currently unknown and needs to be evaluated.

Because of the consistent narrowing associated with the unmeshed bolster, 10 additional pigs were subsequently studied with a 1:1 meshed version of AlloDerm to evaluate whether this type of bolster would be less restrictive on anastomotic dilation. Compared to the unmeshed bolster group, the meshed anastomoses had a marginally larger circumference at 2 weeks, but continued to have significantly smaller circumferences, compared to the unbolstered anastomoses. These data demonstrate that meshing the bolster to a 1:1 ratio does not significantly improve anastomotic dilation early after surgery, though since there was a small increase in size with meshing, it is possible that meshing the tissue, to a greater degree, would further decrease restriction on tissue dilation. The differences in anastomotic dilation between proximal and distal bolster location (as the distal unmeshed bolstered anastomoses did not dilate as much as the proximal unmeshed group, and the proximal meshed bolstered anastomoses did not dilate as much as the distal group) is difficult to explain.

Biochemical analysis revealed that meshed AlloDerm did not affect the content of MMP-2 and -9 in an anastomosis. The similar concentrations of MMP between bolstered and unbolstered anastomoses may reflect the manner in which AlloDerm is processed and the host's reaction to it. AlloDerm's processing allows for, and encourages, tissue ingrowth and incorporation, and there is no foreign-body reaction to the graft material. MMP is an enzyme present in tissue remodeling and is also seen in higher concentrations in inflammatory processes. Because the graft material is washed in a manner that allows the revascularization and ingrowth of host cells, the AlloDerm bolster is incorporated as part of the anastomosis, rather than being encapsulated or attacked as a foreign body and sloughed. While it would not be expected that MMP concentrations of bolstered anastomoses would be less than their unbolstered counterparts so soon after surgery, the fact that MMP concentrations are not elevated by comparison suggests that AlloDerm does not induce further inflammation or tissue reaction, which would be deleterious to normal healing. Though the presence of MMP inhibitors was not addressed in this study, they can also affect MMP concentrations.

As with MMP concentrations, elastin and collagen content were not significantly different between the two types of anastomoses. The opposite finding might have been expected, since AlloDerm is an acellular dermal matrix that contains collagen and elastin. The meshed bolster that was used was no thicker than 0.51 mm, and the thin size of the meshed bolster may have reduced the additional collagen and elastin contributed by the bolster to an insignificant amount. Differentiating between native and allograft collagen and elastin was not performed, as this type of analysis is extremely difficult and fraught with inaccuracies. It should be remembered that the main benefit of AlloDerm is not in providing all of the collagen and elastin needed in the healing and repair process, but rather in providing a specially prepared extracellular matrix consisting of these substrates, which allows host tissue ingrowth and the subsequent creation of the body's own tissue.

The equivalent biochemical composition between meshed bolstered and unbolstered anastomoses provides a biochemical explanation of the equal strength observed with each type of anastomosis. Further, the smaller anastomotic circumferences noted in all bolstered anastomoses do not appear to be related to inflammation caused by AlloDerm or the constitution of AlloDerm, based on our biochemical analysis. The restriction of anastomotic dilation may be a mechanical phenomenon, where the presence of a thin, but nonexpansile, tissue bolster prevents the same degree of dilation of the anastomosis as seen in the absence of a bolster. Longer follow-up may result in further dilation of the bolstered anastomosis, and comparison of bolstered to unbolstered anastomoses further out from the time of surgery would be necessary to know what the final effect on circumference is from various preparations of AlloDerm.

There was no significant difference between the presence of adhesions involving meshed AlloDerm and unbolstered anastomoses. The placement of staples by an EEA device does allow for small gaps between adjacent staples, where the tissue is not perfectly coapted with a watertight seal early after the creation of the anastomosis. Though these small gaps are usually not of sufficient size to cause a symptomatic leak, their presence early in tissue healing may allow for the translocation of a small amount of enteric contents that could lead to a local inflammatory process and subsequent adhesion formation around the unbolstered anastomoses. The presence of AlloDerm between the apposed bowel segments in the bolstered groups may fill these tissue gaps. The overhang of the AlloDerm outside the anastomotic shoulders was minimized but not completely eliminated. This may explain the persistence of adhesions involving the bolstered anastomoses. It is also possible that meshing the AlloDerm prevents the complete filling of the gaps left by the staples.

Summary

The use of unmeshed AlloDerm as a sandwich bolster in end-to-end stapled rectorectal anastomoses appears to produce a significant improvement in anastomotic strength but may cause stricturing at the staple line. The use of a 1:1 meshed AlloDerm tissue bolster represents an attempt at improving on the results of bolstered anastomoses, particularly in regard to strictures. This study demonstrated that meshing the bolster did not improve the strength of the anastomosis, when compared to unbolstered anastomoses, and that the unbolstered anastomoses were already of sufficient strength so that any additional fortitude offered by the bolster was not of statistical or clinical significance. Despite meshing the AlloDerm, the circumference of the bolstered anastomoses continued to be consistently narrower than unbolstered anastomoses, though it did not appear to clinically affect the animals out to 2 weeks from the time of surgery in terms of the advancement of diet and bowel habits. Biochemically, there was no difference created by the presence of a bolster. MMP concentrations were similar between both types of anastomoses, indicating that the AlloDerm was not proinflammatory. Elastin and collagen content were also comparable, providing a biochemical basis for the similar burst strengths that were observed with the meshed bolster. The similar biochemical results reflect that in this setting, the bolster functions as tissue scaffolding and not as a supply of extra matrix material. Adhesions were similar between the two types of bolstered anastomoses. There were no complications with the use of AlloDerm as a bolster.

Conclusions

The routine use of unmeshed and 1:1 meshed AlloDerm bolsters is safe and does not appear to inhibit healing in elective colorectal surgery on healthy subjects. The application of AlloDerm as a bolster in emergent surgery or in patients with factors that are adverse to healing (e.g., use of steroids, irradiated tissue, active infection, or shock) should be further pursued. Until further studied, AlloDerm tissue bolsters should not replace established surgical principles, such as a diversion in a patient at high risk for an anastomotic leak.

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