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Tensile Strength Comparison of Small Intestinal Submucosa Body Wall Repair[1]

Rainier Ko, D.V.M., M.S.,* Evelyn A. Kazacos, D.V.M., Ph.D.,† Scott Snyder, Ph.D.,‡ David M.J. Ernst, B.S.,§ and Gary C. Lantz, D.V.M.*,[2]

**Department of Veterinary Clinical Sciences, †Department of Veterinary Pathobiology, Purdue University, West Lafayette, Indiana; ‡MEDInstitute, West Lafayette, Indiana; and §Cook Biotech Incorporated, West Lafayette, Indiana*

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Background. **Porcine small intestinal submucosa (SIS) has been studied for body wall repair. However, the best method to implant the biological material has not been investigated. The objective of this study was to compare tensile strengths achieved after healing when SIS was placed using three implant techniques (onlay, inlay, underlay) in a porcine model of abdominal wall defect.**

Materials and methods. **Twenty female domestic pigs had three abdominal midline sites assigned to one of five test groups: SIS implantation using inlay, onlay, or underlay technique; sham surgery (sutured midline incision) or normal body-wall control. Full-thickness muscle/fascia midline abdominal defects (6 × 4 cm) were surgically created and then repaired using eight-layer SIS. Healing was evaluated at 1 and 4 post-operative months by tensile strength testing and histopathology.**

Results. **Hernias were not observed. Tensile strengths were not statistically different between the five test groups ($P = 0.39$) or between months 1 and 4 ($P = 0.35$). The caudal site was stronger than the cranial or middle sites in the 1 month group ($P < 0.0001$). Histologically, healing appeared to progress over time as the repair site showed remodeling towards an interlacing fibrous connective tissue pattern.**

Conclusions. **No significant differences in tensile strength were found between implant techniques and were not statistically different from sham surgery and normal control tissue. This study suggested that SIS healing/remodeling provides sufficient tensile strength for the repair of ventral (anterior) abdominal wall defects when implanted using any of three common techniques.**

Key Words: **body wall repair; small intestinal submucosa; tensile strength; hernia.**

INTRODUCTION

Reconstruction of abdominal wall hernias remains a surgical challenge [1–5]. A prosthetic material is necessary when adequate autogenous musculofascial tissue is not present for tension-free closure. Polypropylene is currently the most widely used material [1, 6, 7]. However, the search continues for the ideal prosthetic material [6, 8]. The body wall repair device should meet several criteria that have been previously described [6, 9–11]. The material should allow host tissue incorporation for fixation and a strong, lasting repair, without encouraging scarring and encapsulation, potentiating infection, or inciting a foreign body response [6]. In addition, materials should possess good handling characteristics, be non-carcinogenic, easy to sterilize, and possess adequate strength [11].

Numerous studies of body wall repair devices have been generated by an ongoing search for the ideal prosthetic material [1, 2, 8, 11–14]; however, all clinically available materials have their drawbacks. Complications associated with synthetic non-absorbable materials such as wound infection, bowel fistulae, adhesion formation, seroma formation, and mesh extrusion have been reported [2, 6, 15–19]. Available absorbable synthetic materials include polylactide, polyglactin 910, and polyglycolic acid [6, 20]. Host reaction to these materials has resulted in the deposition of undifferentiated

[1] Supported by Cook Biotech Inc., West Lafayette, Indiana 47906.
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[2] To whom correspondence and reprint requests should be addressed at Department of Veterinary Clinical Sciences, Purdue University, 1248 Lynn Hall, West Lafayette, IN 47907. E-mail: lantzg@purdue.edu.

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and poorly organized tissue [11]. The weak and loosely arranged collagen fibers that remain after the absorbable material is gone may lead to repair failure [6].

Small intestinal submucosa is an acellular, porcine-derived, collagen-based extracellular matrix that has been evaluated as an abdominal wall repair device in rat and dog models [8, 11, 12, 14]. In addition, SIS has been used in a variety of additional applications including vascular grafts [21, 22], dural replacement [23], urinary bladder augmentation [24], perineal hernia repair [25], and other uses [26–29]. The material supports rapid host tissue ingrowth and fosters cellular differentiation, resulting in healed tissue that resembles original host tissue. This may result, in part, from growth factors present in SIS [30]. Body wall repair studies in the dog and rat have used single layer or multilaminate SIS [8, 11, 12, 14]. Examination of the healing response has shown limited inflammation, deposition of organized connective tissue along the lines of stress, and remodeled repair sites that are smooth and confluent with the adjacent native body wall. However, implantation techniques for SIS placement as an abdominal repair device have not been evaluated.

Three techniques for implanting biomaterials to bridge an abdominal wall defect that cannot be primarily closed have been described [6, 31–33]. These include the onlay, inlay, and underlay techniques. The underlay technique is currently considered the best method because of its relatively low hernia recurrence rates [34–37].

The objective of this short term study was to determine the best technique for implanting an SIS prosthesis for repair of a created ventral (anterior) full-thickness porcine abdominal wall (muscle/fascia) defect by comparing tensile strengths achieved during healing.

MATERIALS AND METHODS

Overview of Experimental Design

Tensile strength and histopathology were used to evaluate three techniques (onlay, inlay, underlay) for SIS implantation into created abdominal body wall midline defects in a pig model. Two control treatments, sham surgery and normal intact body wall, were also evaluated. Animal care was approved by the Purdue University Animal Care and Use Committee (PACUC).

The abdominal wall defect repair surgeries were performed on 20 female domestic Landrace pigs with body weights ranging from 55.0 to 68.6 kg (59.7 ± 3.2 kg). Ten animals were allocated to each of two time periods: 1 month and 4 months. Because of space limitation on the animal's abdominal midline, each animal received three of the five treatments. This was done such that all combinations of three of five techniques were represented and each combination was randomly assigned to an animal. Treatment methods were randomized within each group of 10 animals to obtain six samples of each treatment for the 1 and 4 month observation periods.

Surgical Procedure, Post-Operative Care, and Evaluation

Anesthesia was induced with a 0.02 mg/kg intramuscular (i.m.) injection of tiletamine/zolazepam (Telazol, Fort Dodge Laboratories, Inc., Fort Dodge, IA) reconstituted with 2.5 ml of ketamine and 2.5 ml of 10% xylazine and butorphanol (0.1-0.3 mg/kg i.m.). Each animal was maintained on isoflurane via endotracheal intubation. Ventilatory support was used during the entire procedure.

Aseptic surgery was performed. Full thickness (muscle/fascia) 6 cm long × 4 cm wide midline abdominal defects were surgically created and were repaired with eight-layer fenestrated multilaminate SIS (Surgisis Gold, Cook Biotech, Inc., West Lafayette, IN) using standard onlay, inlay, or underlay techniques [6, 31–33] and secured in position with 0 Prolene suture. A 3 cm overlap was used for the onlay and underlay. The SIS was rehydrated for 10 min in sterile saline immediately before implantation. A minimum of 5 cm between treatment sites was achieved. The peritoneal/transversalis fascial layer was closed using 3-0 Monocryl in a simple continuous pattern to prevent direct contact of the implant with underlying viscera. This allowed for later tensile testing of implant/body wall healing and eliminated the confounding effects of variable degrees of abdominal adhesions.

For the sham technique, a 6 cm longitudinal full thickness incision was made on ventral (anterior) midline. The external rectus fascia was apposed with simple interrupted sutures using 0 Prolene.

All subcutaneous tissues were routinely closed with 2-0 PDS in a simple continuous pattern. The skin was closed with 2-0 Monocryl with a simple continuous pattern.

Buprenorphine (0.005 mg/kg i.m.) was administered for post-operative analgesia. Ceftiofur (5 mg/kg i.m.) was given prophylactically immediately preoperatively and then repeated in 12 h. Customized spandex domestic swine jackets (Lomir Biomedical, Inc., Malone, NY) were fitted to each subject to minimize contamination and trauma of the incision sites during skin incision healing.

Daily examination involved visual inspection of the abdominal body wall for evidence of seroma, herniation, or infection.

Jackets were removed at approximately 2 post-operative weeks, once the skin incisions appeared clinically healed. Pigs were euthanized at the predetermined times of 1 and 4 post-operative months by pentobarbital (0.78 mg/kg i.v. to effect). The weights of 1 and 4 month pigs at post-mortem were 78.1 ± 3.1 kg and 143 ± 8.7 kg, respectively.

Gross Pathologic Evaluation

Abdominal walls were harvested immediately after euthanasia. The implant sites were inspected on subcutaneous and visceral sides for evidence of pathology. Aerobic and anaerobic culture samples were obtained from implant sites that appeared to be potentially infected.

Histopathologic Evaluation

An approximate 1.0 cm wide transverse strip incorporating the implant site and adjacent bilateral body wall was harvested from the caudal aspect of the implant site and placed in 10% formalin. This was done to preserve the majority of each implant site for tensile strength testing. Representative sections (six per site) were stained with hematoxylin and eosin (H&E) and examined for fibrosis and inflammation, presence of SIS, changes in the skeletal muscle, and any other pathologic alterations. All evaluations were performed by one pathologist who was blinded to treatments.

Tensile Strength Testing

Transverse strips 1.0 cm wide including the implant site and bilateral adjacent body wall were harvested from the central region of each site and packed in containers of refrigerated normal saline placed on ice until tensile testing that occurred within 3 h of tissue

TABLE 1

Summary of Culture Results

Pig #	Month	Site	Procedure	Other complications	Organism(s) cultured
4	1	Middle	Inlay	S, N	No growth
5	1	Middle	Inlay	S, D	*S. aureus*
6	1	Cranial	Underlay	D	*S. aureus*
13	1	Middle	Underlay	D	*S. aureus*
18	1	Caudal	Onlay	D	*S. aureus*
15	4	Middle	Onlay	N	*A. lwoffii, A. pyogenes*

Note: Culture results shown include seromas as well as delaminated samples with gross evidence of greenish fluid between implant layers; S, seroma; D, delamination; N, non-delaminated.

harvest. Any remaining polypropylene suture material was removed. Sample ends were frapped [38] for 3 to 5 cm with one layer of cotton cloth secured with braided 50 pound test fishing line for better gripping by the testing machine. Both ends of the samples were then placed into grips of the tensile strength testing apparatus (Fast track 8840 Series Dynamight, Norwood, MA). The samples were elongated until failure at a constant rate (100 mm/min). Load and displacement were recorded and load at failure determined. Tensile strength was calculated by dividing the sample load at failure by the sample width. Average tensile strength was determined for each site. The failure mode for each sample was recorded.

Statistical Analysis

The assignment of pigs to treatment groups and the order of individual surgical treatments were determined using an incomplete randomized block design [39]. This experimental design strategy was selected so as to not confound differences in tensile strength among the three suturing techniques and the two controls with anticipated differences in tensile strength among animals.

Tensile strength measurements were compared using a mixed effects Analysis of Variance (ANOVA) model [40]. Significant overall effects were followed by multiple comparisons using Tukey's procedure [39]. A P value below 0.05 was used to establish statistical significance.

The distribution of failure modes was assessed for significance with Fisher's Exact Test, and failure mode was incorporated into the analysis of tensile strength measurements using Kaplan-Meier estimates of the tensile strength at failure, for specific failure modes.

All analyses were performed using SAS Enterprise Guide version 3.0, running SAS version 8.2 (SAS Institute, Cary, NC).

RESULTS

Clinical Evaluation

Surgery, anesthesia, and recovery were uneventful in all animals. Small to moderate seromas, diagnosed by observation and palpation, developed in 10% (2/20) of animals and 4.2% (2/48) of defects. Both seromas occurred in the 1 month group. There were no clinical indications of implant failure evidenced by palpation or bulging of surgery sites in any pig during the postoperative observation period.

Gross Pathology: One Month

The boundaries of the repair device were easily identified. The repair site appeared smooth, pliable, and confluent with the adjacent musculo-fascial tissue. No gross abnormalities were noted in the sham or normal specimens. Intra-abdominal adhesions and herniation were not present at the implant sites. Separation of SIS layers (delamination) that indicated incomplete incorporation of the SIS was noted in 38.9% of 1-month implant specimens (three inlay, one onlay, and three underlay). A thick greenish fluid found within the caverns of the delaminated SIS was present in 22.2% (4/18) of the 1 month implant sites. Aerobic and anaerobic culture samples were obtained from these sites and from the two sites that developed seromas. Infection was documented in four implant specimens (two underlay, one inlay, one onlay) and one seroma (Table 1). Four of 18 (22.2%) SIS implant sites were infected at 1 month.

Histopathology: One Month

No abnormalities were noted in the clinically normal body wall specimens. Sham surgery sites showed fibrous connective tissue arranged in an interlacing bundle pattern.

Two collagen organization patterns were noted. One pattern consisted of SIS replacement with fibrous connective tissue that was infiltrated with mononuclear inflammatory cells (Fig. 1). Only a small amount of SIS material remained. This reaction pattern was observed in two inlay, five onlay, and two underlay specimens.

The second histological pattern consisted of separation of layers of remaining SIS material by fibrous

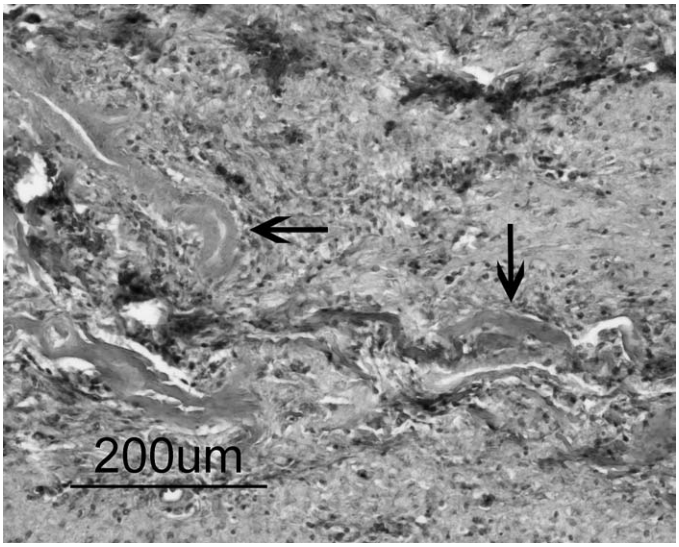

FIG. 1. Inlay implant site at 1 month in pig #12. The SIS material is largely replaced by abundant fibrous connective tissue that is infiltrated with mononuclear inflammatory cells. Scant remnants of SIS appear as acellular, hyaline, wavy material (arrows).

connective tissue with mononuclear inflammatory cells (Fig. 2) between the layers. This reaction pattern was observed in four inlay, one onlay, and four underlay specimens.

Samples examined from infected sites (Table 1) found SIS layer separation by fibrous connective tissue with degenerating neutrophils.

Gross Pathology: Four Month

The SIS implant was no longer discernable from the fibrous tissue matrix. Intra-abdominal adhesions and herniation were not seen at the implant sites. In contrast to the 1 month implant specimens, only one underlay sample (5.3%) showed evidence of minimal delamination. One non-delaminated implant site also had greenish fluid in the adjacent subcutaneous tissue and was infected (Table 1). One of 18 (6.8%) of SIS implant sites were infected at 4 months.

Histopathology: Four Month

Histopathologic alterations were not present in the normal body wall specimens. All sham specimens had fibrous connective tissue arranged in interlacing bundles as was found at 1 month.

All SIS implant sites consisted of dense fibrous connective tissue and many had a minimal to mild mononuclear inflammatory cell infiltrate throughout the connective tissue (Fig. 3). The fibrous connective tissue appeared as interlacing bundles in three inlay, five onlay, and four underlay specimens (Fig. 4). Small-scattered remnants of SIS were observed in only two implant specimens (one inlay and one underlay).

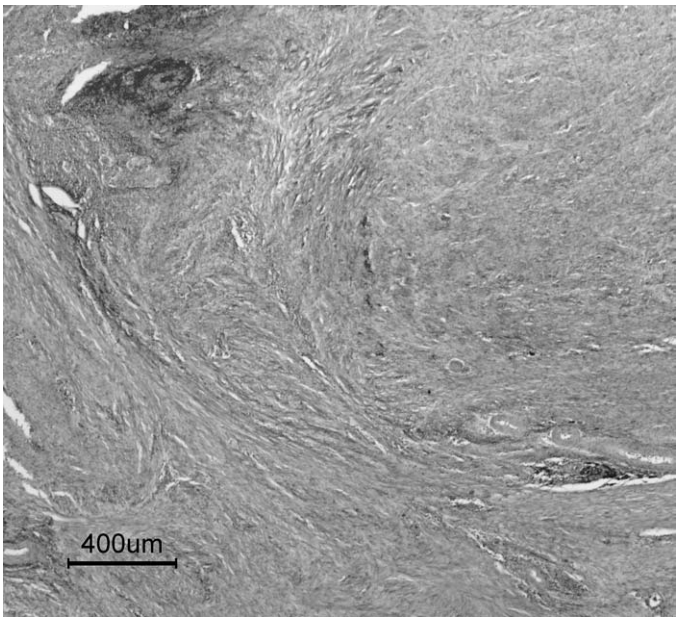

FIG. 3. Underlay implant site at 4 months in pig #8. The SIS has been replaced by fibrous connective tissue arranged in broad sheets rather than in an interlacing pattern.

Tensile Strength

Table 2 presents the mean failure load per centimeter width by technique and time period. ANOVA modeling allowed testing for differences between the time periods, the sample location, and treatments. The *P* values for these effects are listed in Table 3. The month:site and site:procedure interactions were significant ($P = 0.03$ and 0.02, respectively). For the month:site interaction, within month 1, the caudal site was

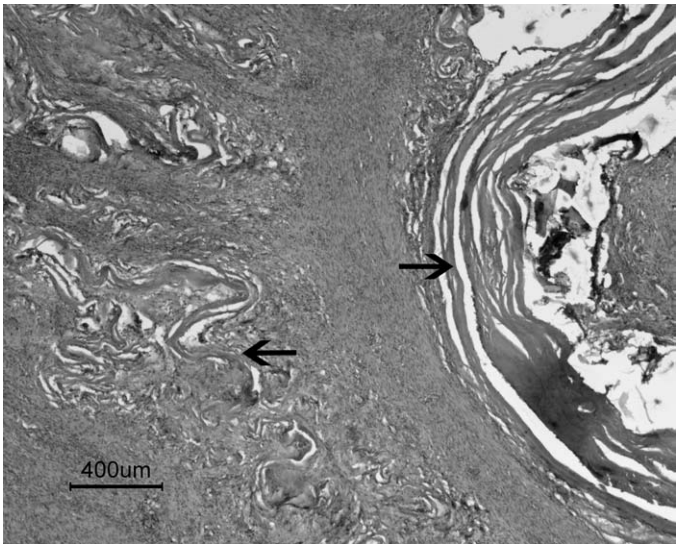

FIG. 2. Underlay implant site at 1 month in pig #17. Abundant, delaminated SIS (arrows) is separated by fibrous connective tissue infiltrated with moderate numbers of mononuclear inflammatory cells.

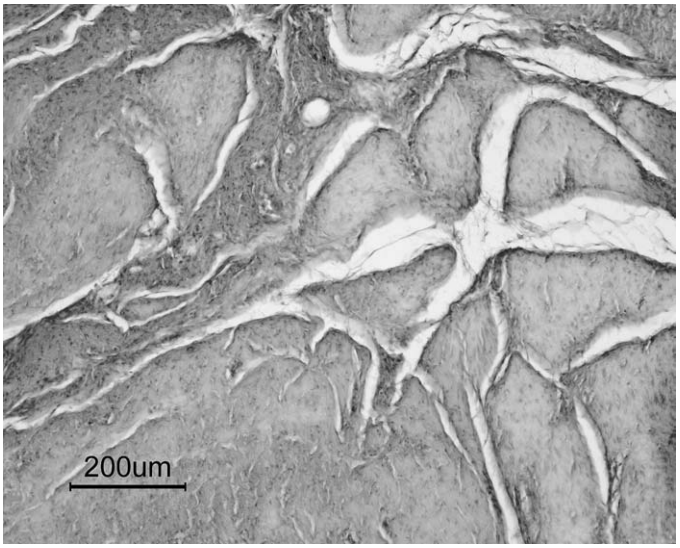

FIG. 4. Inlay implant site at 4 months in pig #2. The SIS has been replaced by fibrous connective tissue forming an interlacing pattern similar to that seen in the sham.

TABLE 2

Summary of Mean Tensile Strength (N/cm* ± SD†) by Month and Technique

	1 month			4 month		
Technique	Animals	Samples	Mean ± SD† (N/cm*)	Animals	Samples	Mean ± SD† (N/cm*)
Underlay	6	18	54.8 ± 25.7	6	12	54.2 ± 28.0
Inlay	6	12	65.7 ± 29.4	6	12	47.3 ± 28.8
Onlay	6	15	48.3 ± 18.0	6	14	65.4 ± 28.0
Sham	6	9	53.1 ± 21.6	6	8	74.8 ± 45.4
Normal	6	14	44.7 ± 15.4	6	11	41.3 ± 36.0

Note: * N/cm, newtons per centimer; † SD, standard deviation.

significantly greater than the cranial and middle sites ($P = 0.001$ and 0.007, respectively). This pattern was not present at month 4. For the site:procedure interaction, the mean tensile strength of the caudal/underlay combination was significantly greater than the cranial/inlay, cranial/normal, cranial/sham, and middle/underlay combinations ($P = 0.021$, <0.001, 0.006, and 0.001, respectively). Furthermore, the caudal/sham combination was significantly greater than cranial/normal and middle/underlay combinations ($P = 0.011$ and 0.029, respectively). Analysis of the tensile strength data revealed that across site and month, all surgical techniques were statistically indistinguishable from sham control or normal body wall.

Tensile Strength Analysis in Conjunction with Failure Mode

Table 4 presents the six modes of failure that occurred during tensile strength testing. Few failures occurred at the repair site (implant or fascia/implant junction). The majority of the failures occurred in a region of the sample that was unrelated to the implant or fascia/implant junction. Failures at the testing machine grip and the fascia accounted for 86.8% and 80.7% of the failures at 1 and 4 months, respectively. The distribution of failure mode was significantly different between months 1 and 4 ($P < 0.0001$). The statistical significance was attributed to more failures occurring at the grips of the testing device for the 4 month samples.

Evaluation of implant and implant-fascial junction failure using Kaplan-Meier survival curves found no difference in the tensile strength failure distribution between the two time periods ($P = 0.82$) (Fig. 5) or between the five test techniques ($P = 0.27$) (Fig. 6).

The Kaplan-Meier plots confirm that no difference in the tensile strength failure distributions existed between time periods, between surgical techniques, and between surgical techniques and normal body wall.

DISCUSSION

The current study did not show a statistically significant difference in tensile strength between surgical technique groups and normal body wall, between the 1 and 4 month time periods. These results suggested that all repair methods were sufficiently strong during the course of this study to maintain abdominal wall integrity.

Degradable biomaterials provide certain advantages over non-degradable biomaterials. Degradable materials are less susceptible to perpetuate infection and tend to cause less foreign body response [6, 41]. It is

TABLE 3

Statistical Significance* (*P value*) for Tensile Strength

Source	*P* value
Month	0.35
Site	<0.0001*
Procedure	0.39
Month:Procedure	0.23
Month:Site	0.03*
Site:Procedure	0.02*
Month:Site:Procedure	0.19

Note: ANOVA modeling required a natural log transformation of tensile strength values; * $P < 0.05$.

TABLE 4

Failure Mode for Tensile Strength Testing

Failure mode	1 month	4 month
Upper or lower grip specimen slippage	3 (4.4%)	27 (47.4%)*
Fascia between grip and repair	56 (82.4%)	19 (33.3%)
Implant/fascia junction	2 (2.9%)	3 (5.3%)
Implant	7 (10.3%)	6 (10.5%)
Unknown	0 (0%)	2 (3.5%)
Total	68	57

Note: Data presented in Table 4 represents the occurrences (followed by percentage) for a given failure mode at 1 and 4 month time periods. Few failures occurred at the implant or implant/fascial junction. More failures occurred at the fascia between the grips and repair site at both time periods. * $P < 0.0001$.

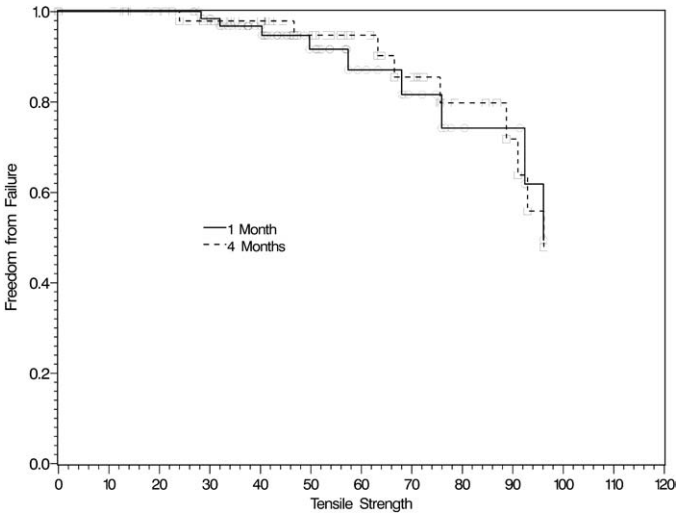

FIG. 5. Kaplan-Meier plot of freedom from failure at the repair site versus tensile strength for 1 and 4 month time periods. Tensile strength is expressed in Newton per centimeter. No significant difference in the tensile strength failure distribution between the two time periods was found ($P = 0.82$).

important to understand the biological response to degradable biomaterials and the expected mechanical properties of the implant and replacement tissues over time for various clinical applications. In a canine abdominal wall defect study, Badylak showed that SIS had biaxial tensile strength properties that changed following *in vivo* implantation depending on the balance between the rate and degree to which simultaneous replacement of the biomaterial by host tissue occurred [42]. The strength of eight-layer implanted SIS was shown to decrease to its nadir at 10 days post-surgery, then progressively increased to over double its original strength at implantation by 24 months. The ultimate load bearing capability of the repair site exceeded that of the native tissue at all time points.

In the current study, repairs at the caudal site at 1 month appeared to be significantly stronger than other locations. These results are difficult to explain. Combining information from porcine and human anatomy references with the observations in this study, the abdominal wall organization appeared to be very similar in both species [43–48]. In the caudal portion of the abdominal wall, pigs seem to resemble humans in that the rectus muscle lacks an internal aponeurotic layer, being covered in this region by only a thin continuation of the transversalis fascia and peritoneum [43]. It is unlikely that this anatomical difference may explain the significantly higher strength values found at this site compared to the cranial or middle sites. Statistical verification of these results may be performed by increasing the sample size. An evaluation of risk factors in incisional hernia recurrence in man failed to show a statistical difference in the location of the surgical repair [49].

Failures within the repair site (implant and/or implant/fascial junction) are an indicator of the tensile strength of the surgical repair. Most tissue failures in this study occurred in the fascia between the testing grip and repair site at both time periods. This type of failure mode indicates a lower tensile strength of fascia than at the repair site. The primary difference was substantially more failures occurring at the grips of the testing device at 4 months. This is most likely explained by technical difficulties of fitting and securely holding the significantly thicker 4 month samples in the grips of the tensile strength testing apparatus. The mean weights of the 4 month porcine subjects were substantially greater than their 1 month counterparts (143.0 $\pm$ 8.7 kg *versus* 78.1 $\pm$ 3.1 kg, respectively). In addition, failure modes at either the upper or lower clamps implied that the repair site was stronger than the tensile strength value recorded for that sample. These failures indicate a technical limitation imposed by the testing apparatus.

The position of the implanted prosthesis is thought to play an important role in hernia recurrence [6, 50]. Since the original description by Rives in 1973 and development by Chevrel, Wantz, and Stoppa, the underlay technique has come to be widely used for open mesh repair resulting in the lowest rates of hernia recurrences [34–37]. The advantage of this technique is the position of the implant behind the rectus muscles where the force of abdominal pressure holds the prosthesis against the deep surface of the abdominal muscle wall. Alternatives for the more technically challenging underlay technique are the onlay [6, 33] and inlay techniques [6, 31]. They have the disadvantage of lacking fixation of the implant by intra-abdominal pressure

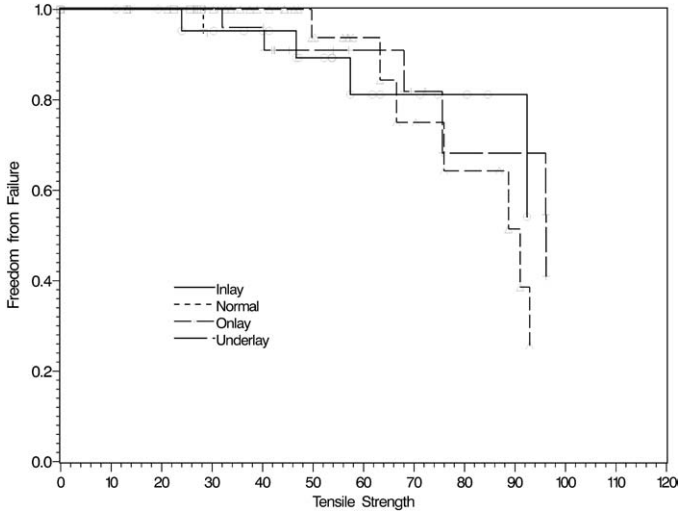

FIG. 6. Kaplan-Meier plot of freedom from failure at the repair site *versus* tensile strength for operative techniques. Tensile strength is expressed in Newton per centimeter. Sham control surgery was not represented since no failures occurred at the repair site. No significant difference in the tensile strength failure distribution between the techniques was found ($P = 0.27$).

that may lead to a relative lack of mechanical support and contact by overlying tissues. Inlay also involves minimal surface area of contact (essentially edge-to-edge) between the implant and the adjacent fascia. This may lead to insufficient anchorage of the implant. The present study did not support the notion that implantation technique significantly alters the tensile strength of the healing repair. However, results of this quadruped model used in the current study may differ from bipeds with respect to the distribution of forces along the abdominal wall and repair sites.

Tensile strength findings in this study suggest that the rate of incorporation of SIS by the host may minimize the importance of a broad tissue surface area of implant contact as is strived for in the onlay and underlay techniques. This observation must be tested in human clinical studies. Rapid SIS implant incorporation was found in other animal studies of abdominal wall reconstruction using SIS [8, 11, 12, 14].

Wound complications, such as hematoma, seroma, and infection are reported in 0 to 36% of patients after abdominal wall hernia repair [31]. The onlay procedure is often associated with higher complication rates [31, 51–56]. This may be because of the extensive dissection necessary to separate the skin and subcutaneous tissue from fascia to allow for adequate implant and fascia overlap. This large dissection surface area may predispose to seroma formation. Separation of the epigastric perforating arteries may interfere with wound healing and may increase the risk of infection. In the current study, the most common complication was incomplete incorporation (delamination) of the SIS implant among the 1 month group as compared to the 4 month group. This can most likely be attributed to further remodeling and incorporation of SIS in the repair site over time.

When implanted into soft tissue, SIS typically induced a mononuclear inflammatory response that appeared to subside after the first month [12, 21, 22]. Single or double layer SIS was used in these studies and it is logical to assume that more rapid capillary penetration of these thinner SIS implants resulted in earlier remodeling and complete host incorporation than was apparent in the present study. Multiple implants in the current study, particularly at 1 month, consisted of focal, cavitating lesions with separation between the SIS layers. This pattern was characterized by a strong mononuclear or degenerative neutrophilic infiltration of inflammatory cells within and surrounding the repair site. It is possible that this inflammatory response was related to the lack of incorporation of the SIS and subsequent deterioration of the more centrally located non-incorporated layers of SIS. Although microbial agents were not observed, the presence of an intense degenerative neutrophilic inflammatory response may be indicative of reaction to the implant or a surgical site infection. It seems likely that the biological fate of the SIS implants was eventual incorporation into the fibrous tissue repair matrix, as evidenced by the absence of delamination among all but one 4 month sample.

The nature of both histological responses seen in 1 and 4 month groups were different than those observed in a previous study [12]. In a canine lateral abdominal wall defect model, double layer SIS was totally replaced by organized collagenous tissue by 4 months [12]. The resulting remodeled collagen formed organized sheets aligned in a direction parallel to that of the adjacent fascia along the lines of stress. In the current study, the fibrous connective tissue in both the 1 and 4 month groups often showed a distinctive interlacing bundle pattern. This histological architecture is similar to normal midline aponeuroses and reflects remodeling toward normal fascia. This may also reflect healing on the midline as opposed to healing of the lateral abdominal wall or may be related to implantation of an eight-layer SIS implant as opposed to a two-layer implant. At four months, remaining SIS was rarely observed within the fibrous connective tissue.

Samples for bacterial culture were not routinely obtained from all implant sites. Each seroma was cultured and only one yielded positive results. Cultures were obtained from implant sites that contained a thick mucoid consistency green material in areas of delamination within 1 month SIS implants and one non-delaminated 4 month implant. It is possible that the implant sites may be more susceptible to infection early in the healing/remodeling phase when the implant is still in the early phase of incorporation by the host and the central layers of the implant remain isolated from penetrating capillaries.

Potential sources of infection include surgical contamination and/or entrapment of circulating bacteria in the non-vascularized inner layers of the SIS implant. Infections in this pig model may also be related to the housing environment, suture line trauma induced by normal porcine behavior, and potential bacterial migration around the skin sutures. Although the stalls and protective jackets were kept clean, the environment was not ideal for a healing incision site. Open surgical repair without post-operative wound drainage may also have contributed to the infection rate.

Implantable prostheses, such as expanded polytetrafluoroethylene (PTFE) (Gore-Tex Soft Tissue Patch; W.L. Gore & Associates, Inc., Flagstaff, AZ) or polypropylene mesh, have infection rates that range from 0.5 to 7.7% [1, 57–59]. Placement of permanent mesh in heavily contaminated fields has been found to result in infection rates as high as 50 to 90% [60]. Experimental vascular graft studies using single and four layer SIS demonstrated apparent resistance to infection [61–63]. Franklin *et al.* investigated the use of 4 and 8 layer SIS mesh in laparoscopic ventral hernia repair in contam-

inated fields in humans and reported no complications or infections within 2 years of follow up [64]. In contrast, Ueno *et al.* reported a post-surgical wound infection rate of 47% in patients undergoing open ventral hernia repair using 8 layer SIS in the presence of bacterial contamination [65]. Helton *et al.* further supported these findings and stated that increased wound complications and mesh infections in open surgeries may be related to more intense wound inflammation associated with an incision and closure when compared with the laparoscopic mesh repair [66]. It was also concluded that SIS in critically ill patients with contaminated wounds warranted caution [66]. These studies suggest that local wound environment and rate of complete incorporation by the host may be important factors in the complication rate.

Holtom *et al.* showed that SIS did not exhibit innate antimicrobial properties *in vitro* [67]. Differences in infection rates may, therefore, be related to the SIS implant thickness that may affect the rate and capillary penetration and subsequent incorporation. Eight layer SIS used in this porcine study revealed incomplete incorporation at one month and may have a similar trend in humans [66]. The rate of eight-layer SIS implant incorporation may interfere with wound remodeling and serve as a nidus for infection.

The results of the present study show that all implantation techniques resulted in repairs that were at least as strong as the normal porcine body wall. The SIS bioscaffold became progressively incorporated into the body wall with a histological appearance approximating normal fascia by 4 post-operative months. Continued investigation of the balance between adequate implant tensile strength at the time of surgery and the rate of complete implant incorporation over time is needed to possibly reduce infection potential by promoting more rapid incorporation and remodeling.

We conclude that all three implantation techniques result in similar tensile strengths at 1 and 4 months after operation and that the tensile strengths after implantation are not statistically significant from the sham surgery and normal control abdominal body wall. This study suggested that SIS demonstrated sufficient tensile strength and healing/remodeling for the repair of ventral (anterior) abdominal wall hernias when implanted by three commonly used techniques. These results must be verified in human clinical studies.

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