

Evaluation of intraperitoneal placement of absorbable and nonabsorbable barrier coated mesh secured with fibrin sealant in a New Zealand white rabbit model

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Abstract

Background This study aimed to evaluate the acute and chronic fixation strength of fibrin sealant (FS) as an alternative method of fixation for laparoscopic ventral hernia repair (LVHR).

Methods Representative mesh types for LVHR included one nonabsorbable barrier mesh (Composix) and three absorbable barrier meshes (Sepramesh, Proceed, and Parietex composite). Macroporous polypropylene mesh (Prolite Ultra) served as the control mesh. Three methods of fixation were used, namely, 0-polypropylene suture + FS (ARTISS 4 IU), FS alone (ARTISS), and tacks alone, to secure 3 × 4-cm pieces of mesh (10 of each combination) to the peritoneal surface of New Zealand white rabbit abdominal wall. After 2 h of incubation at 37°C, specimens underwent acute testing. Subsequently, a chronic phase was completed using the aforementioned fixation methods (10 of each combination), in which two 4 × 4-cm pieces of mesh were secured intraperitoneally in each of 75 New Zealand white rabbits, which survived 8 weeks until they were sacrificed. A transparent grid overlay was used to measure the mesh and adhesion area. Adhesion tenacity was characterized using the Garrard adhesion scale. In both the acute and chronic samples, a 3 × 3-cm area of mesh–tissue interface underwent lap shear testing at a rate of 0.42 mm/s using a tensiometer (Instron 5542). The maximum load sustained by the

mesh–tissue construct was recorded as the acute fixation strength in newtons (N). Data are given as means ± standard error of the mean. Statistical significance ($p < 0.05$) was determined using a one-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) posttest or a nonparametric Kruskal–Wallis test (adhesion scores).

Results The acute fixation strength was significantly greater for all the meshes secured with either suture + FS or tacks alone than for FS alone ($p < 0.001$ for all comparisons). All the meshes except Proceed demonstrated greater acute fixation strength with suture + FS than with tacks alone ($p \leq 0.016$). Composix achieved greater acute fixation with suture + FS than all the other meshes ($p \leq 0.022$). Acute fixation with suture + FS was greater for Parietex Composite and ProLite Ultra than for Proceed ($p \leq 0.015$). When the animals were sacrificed, 48 of 50 meshes fixed with FS alone were insufficiently affixed to the abdominal wall, which may have resulted in hernia recurrence in a hernia model. The chronic fixation strength was greater for all the mesh types with either suture + FS or tacks only than with FS alone ($p \leq 0.0005$). The chronic fixation strength was greater with suture + FS than with tacks for Proceed and ProLite Ultra ($p \leq 0.013$). Neither mesh area nor adhesion tenacity differed significantly with any mesh/fixation method combination.

Conclusions In a chronic rabbit model of LVHR, fixation strength with FS alone was inadequate for selected non-absorbable and absorbable barrier-coated meshes. The acute and chronic fixation strengths of suture + FS were equivalent or superior to the fixation strength of tacks alone. Using a combination of suture and FS for mesh fixation in LVHR may provide adequate fixation while decreasing postoperative pain due to spiral titanium tacks. In this preclinical series, mesh secured to the peritoneal surface by FS alone may have led to early recurrence.

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Current fixation techniques using permanent suture with or without tack fixation in laparoscopic ventral hernia repair (LVHR) are associated with better outcomes than open techniques [1]. However, significant acute postoperative pain and chronic pain syndromes are associated with trans-fascial sutures [2, 3], and intraperitoneal staple or tack fixation constructs can potentially cause intestinal adhesions, obstruction, erosion, or fistulization [2, 4–6]. Thus, research to determine the optimal method of mesh fixation in LVHR continues, including investigations into the use of absorbable fixation devices and fibrin sealant (FS) products.

In 1997, Chevrel and Rath [7] described the first use of FS products in a series of onlay hernia repairs in which FS was used as an adjunctive method of mesh fixation. Since that time, FS products have been used in an increasing number of hernia applications.

An FS product, TISSEEL/TISSUCOL (Baxter Healthcare Corp, Deerfield, IL, USA), manufactured with a 500 IU/ml thrombin concentration, has been used as an alternative fixation method in multiple laparoscopic transabdominal preperitoneal (TAPP) and totally intraperitoneal (TEP) series [5, 8–13]. Another formulation of FS, ARTISS (Baxter Healthcare Corp), contains a lower concentration of thrombin and received Food and Drug Administration (FDA) approval in 2008 for the purpose of skin graft fixation to burn wound beds [14]. When reconstituted, ARTISS contains a thrombin concentration of 4 IU/ml. Neither product is currently FDA approved for fixation of prosthetic mesh. Nor has ARTISS been evaluated in a model of either preperitoneal or intraperitoneal mesh fixation.

The FS products are biodegradable adhesives. They are packaged as two separate components (human-derived fibrinogen and calcium chloride-activated thrombin), which are mixed at application and form polymerized fibrin chains [2]. The fibrinogen component gives the product its tensile strength [10], and the thrombin concentration affects the rate of fibrin polymerization.

One difficulty with the use of an FS product in a laparoscopic setting is the short time for manipulation of the applied product before polymerization. Surgeons have only 3 s for graft positioning before polymerization of the TISSEEL FS product. In contrast, the reduced thrombin concentration in ARTISS allows 60 s for graft or mesh positioning before polymerization. An FS with lower thrombin concentration may facilitate clinical application in laparoscopic hernia repair if equivalent fixation strength is

achieved while greater time for graft manipulation is allowed before polymerization.

Data have been presented demonstrating equivalent acute fixation strengths ($p > 0.05$) for TISSEEL and ARTISS used with selected nonabsorbable (Composix) and absorbable barrier-coated meshes (Sepamesh, Proceed, and Parietex Composite Mesh) and one nonbarrier macroporous polypropylene mesh (Prolite Ultra) when applied to the peritoneal surface [15]. However, the overall acute fixation strengths were low, with TISSEEL averaging 2.8 ± 0.2 N and ARTISS averaging 4.3 ± 0.3 N (shown as the mean \pm standard error of the mean [SEM] for all the mesh types evaluated) [15]. These low fixation strengths indicate the need for further comparative acute and chronic fixation strength testing. This study therefore aimed to evaluate the acute and chronic fixation strengths of suture + FS, tacks only, and FS only for attachment of absorbable and nonabsorbable barrier meshes to the peritoneum in a rabbit model.

Methods

All study activities were performed under a protocol (#20060254) approved by the Washington University School of Medicine Animal Studies Committee. All the animals were housed, fed, and handled according to established protocols for humane animal treatment currently in practice at our institution. In this study, 92 New Zealand white rabbits weighing 3–4 kg were used. All the animals were sacrificed using intramuscular injection of ketamine (35 mg/kg) and xylazine (6 mg/kg) for sedation followed by an intravenous pentobarbital overdose (>300 mg/kg) before abdominal wall tissue harvest.

Mesh selection

Five mesh types were included in this study. Four mesh types approved by the FDA for intraperitoneal placement and used in LVHR were evaluated. The four meshes included three absorbable barrier meshes—Sepamesh (Genzyme Corp, Cambridge, MA, USA), Proceed (Ethicon Inc., Somerville, NJ, USA), and Parietex Composite Mesh (Covidien)—and one nonabsorbable barrier mesh, Composix (C.R. Bard, Inc., Murray Hill, NJ, USA). A nonbarrier macroporous polypropylene mesh, Prolite Ultra (Atrium Medical Corp, Hudson, NH, USA), was chosen as the fifth mesh type. The meshes were cut or specially manufactured (Composix) to the size of 3×4 cm (Fig. 1).

Acute mesh fixation

For the acute arm of this study, 17 rabbits were obtained as a source of abdominal wall tissue. Three methods of

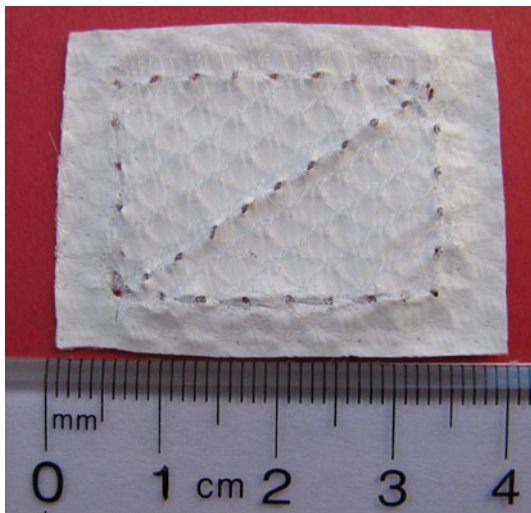


Fig. 1 A 3 × 4-cm piece of Composix manufactured by C.R. Bard, Inc

fixation—suture + FS (ARTISS 4 IU), FS alone (ARTISS), and tacks alone—were used to secure 3 × 4-cm pieces of mesh including Composix, Sepramesh, Proceed, Parietex Composite Mesh, and Prolite Ultra.

Each fixation method was used to secure ten pieces of each mesh (ten of each combination, for a total of 150) to the peritoneal surface of abdominal wall harvested from New Zealand white rabbits. Use of the tacking device (ProTack; Covidien) involved placing two constructs along the shorter edge (3 cm) of the mesh 1.5 cm apart. Samples affixed with suture (0-Surgipro; Covidien) + FS were secured with two sutures placed 1.5 cm apart along the 3-cm edge of the mesh and tied down with eight knots after application of the FS. The FS was reconstituted according to the manufacturer's instructions and maintained at 37°C in a Fibrinotherm (Baxter Healthcare Corp), a heating and stirring device that helps to automate the reconstitution of FS products.

Two separate syringes of fibrinogen mixed with aprotinin and the activated thrombin were prepared and loaded into the dual-syringe system provided in the kit. An even layer of FS was sprayed on the peritoneal side of a 3 × 3-cm area of harvested rabbit abdominal wall using a Duplospray MIS Applicator (Baxter Healthcare Corp). A 3 × 3-cm area of a 3 × 4-cm piece of mesh then was secured to the abdominal wall for both the FS alone and the suture + FS fixation methods. Gentle pressure was applied for 5 min for each sample secured with FS to allow bonding between the mesh, sealant, and abdominal wall. For each piece of mesh, 1 ml of FS was used. All the samples using FS for fixation were maintained at body temperature (37°C) for 2 h before fixation strength testing to allow complete polymerization of the FS.

Chronic mesh fixation

The chronic arm of the study used 75 rabbits. Each rabbit was induced via intramuscular injection of ketamine (35 mg/kg) and xylazine (6 mg/kg). After orotracheal intubation, the animals were positioned supine. Intraoperative general endotracheal anesthesia was maintained with 3% isoflurane. Saline was given intravenously as needed during the operative procedure. Perioperative buprenorphine was administered subcutaneously at a dose of 0.02–0.05 mg/kg, and preoperative prophylactic antibiotics were administered intramuscularly as Baytril dosed at 2.5–5 mg/kg. The skin was prepped and cleansed with 10% povidone-iodine solution (Betadine). The animals were subjected to surgery under strict sterile conditions.

After a 6-cm midline laparotomy incision had been made, a 4 × 4-cm piece of mesh was secured to an intact peritoneum on each side of the incision. The mesh selection, as described previously, included: Composix, Sepramesh, Proceed, Parietex Composite Mesh, and Prolite Ultra. As in the acute testing, the same three methods of fixation were used: suture + FS (ARTISS), FS alone (ARTISS), or tacks alone. The animals were randomly assigned to the method of fixation and mesh selection. With each fixation method, ten pieces of each mesh were secured (ten of each combination, for a total of 150 implanted mesh pieces). In the tacks only group (ProTack) and the suture (0-Surgipro) + FS group, three constructs (either tacks or sutures) were placed along both the medial and lateral edges of the mesh, as shown in Fig. 2. Placement of the constructs in this manner allowed for later division of the mesh for tensile testing and histology (see

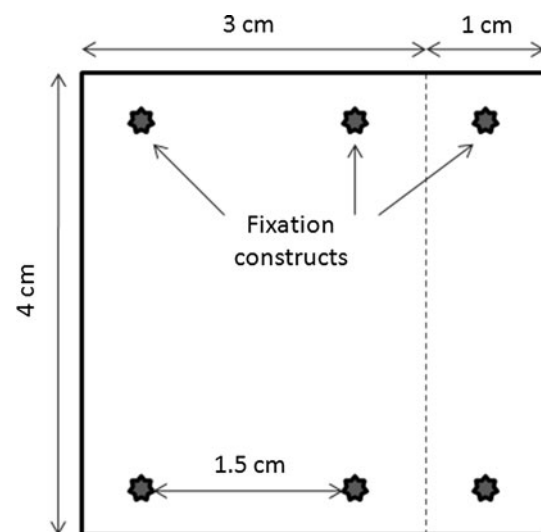


Fig. 2 Graphic depiction of chronic mesh implant dimensions and fixation construct locations. Dotted line depicts later division point for tensiometry and histology samples

dotted line in Fig. 2). Sutures were placed in a transfascial manner and tied down using eight knots after application of the FS, with the knots were buried subcutaneously. For the FS alone and suture + FS fixation methods, the FS was prepared and applied as with the acute methods described earlier except that the FS was applied to a 4 × 4-cm area of intact intraperitoneal abdominal wall for fixation of 4 × 4-cm pieces of mesh. Also, before prepping of the animals' skin, the animals undergoing mesh fixation with FS alone received four skin tattoos to mark the corners of the planned implant location of each mesh piece. At implantation, FS was applied, and the mesh pieces were centered beneath the corresponding tattoo overlays.

After mesh implantation, the abdominal wall fascia and subcutaneous tissue were closed separately with interrupted 3-0 Prolene and 4-0 PDS II (Ethicon Inc) sutures, respectively, and the skin was closed with tissue adhesive. Post-operatively, subcutaneous buprenorphine (0.02–0.05 mg/kg) was given the evening of surgery, the following morning, and then on an as needed basis.

Chronic mesh evaluation

The animals were kept alive for 8 weeks, then sacrificed. A wide circumferential incision was made to allow examination of the entire abdominal cavity, and the mesh samples were inspected visually for adhesions. Any adhered organs were incised away from the mesh surface to allow separation of the abdominal wall from the abdominal cavity.

A transparent grid overlay was used to measure the mesh area and adhesion area for all the mesh samples. Adhesion tenacity was characterized using the Garrard adhesion scale (Table 1). The positions of the mesh secured with FS alone were compared with the exterior abdominal wall tattoos described previously using four needles passed through the entire abdominal wall. This was done to determine whether the mesh had migrated from the original fixation location. All the mesh samples were examined for attachment to the abdominal wall. If the mesh had migrated more than 2 cm from the implant location or was grossly not attached sufficiently to the abdominal wall, it was deemed a clinical failure.

Table 1 Adhesion Grading Scale

Type of adhesions	Score
No adhesions	1
Filmy adhesions, easily broken manually	2
Dense adhesions, requiring blunt dissection to separate viscera from mesh	3
Very dense adhesions, viscera matted to mesh, requiring sharp dissection to separate viscera from mesh	4

The mesh samples were divided into 1 × 4-cm pieces for histology and 3 × 4-cm pieces for fixation testing, as demonstrated by the dotted line in Fig. 2. The histology samples were fixed in 10% neutral buffered formalin. Further histologic evaluation is not described in this report. The fixation testing samples were prepared for testing by removal of the two fixation constructs from one 3-cm edge of the mesh. A free edge of mesh then was developed to allow fixation testing by removal of the attachment to the abdominal wall for a 1 × 3-cm area of mesh.

Fixation strength testing of acute and chronic samples

The fixation strength-testing methods were similar for acute and chronic samples. The mesh–tissue interface underwent lap shear testing using an Instron Series 5542 Materials Testing Machine (Instron, Norwood, MA, USA). Screw-action grips were used to grip the samples during testing. The free edge of mesh, measuring 1 × 3 cm, was secured in the upper grip (Fig. 3). The free edge of abdominal wall tissue was secured in the lower grip. The fixation interface (3 × 3 cm) was tested in tension at a constant rate of 0.42 mm/s until the mesh completely sheared off the underlying tissue. The maximum load sustained by the construct was recorded as either the acute or chronic fixation strength in units of newtons (N), where 1 N equals 0.225 lb (or 1 lb equals 4.4 N). Data are presented as means ± SEM.

Statistical analysis

Statistical significance ($p < 0.05$) was determined using a one-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) posttest or a nonparametric Kruskal–Wallis test (adhesion scores).

Results

Acute fixation strength

The acute fixation strengths for all mesh and fixation combinations are presented in Fig. 4. The acute fixation strengths were significantly greater for all the meshes secured with either suture + FS or tacks alone than for FS alone ($p < 0.001$ for all comparisons). All the meshes except Proceed demonstrated greater acute fixation strength with suture + FS than with tacks alone ($p \leq 0.016$). Composix achieved greater acute fixation with suture + FS than all the other meshes ($p \leq 0.022$). The acute fixation strength with suture + FS was greater for Parietex Composite and ProLite Ultra than for Proceed ($p \leq 0.015$). The acute fixation strength achieved by

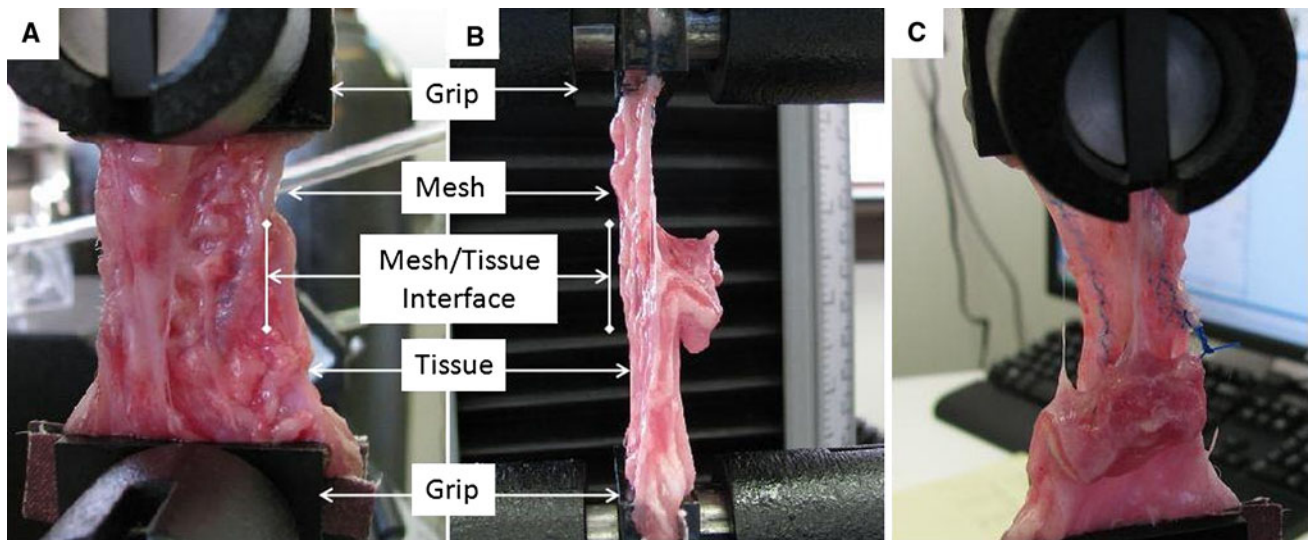


Fig. 3 Chronic mesh sample (Proceed) attached to abdominal wall tissue. **a** Mesh + tissue specimen prepared to undergo lap shear fixation testing. **b** Lateral view during lap shear testing. **c** Tissue view

during lap shear testing depicting the point at which most of the mesh has been sheared off the underlying tissue

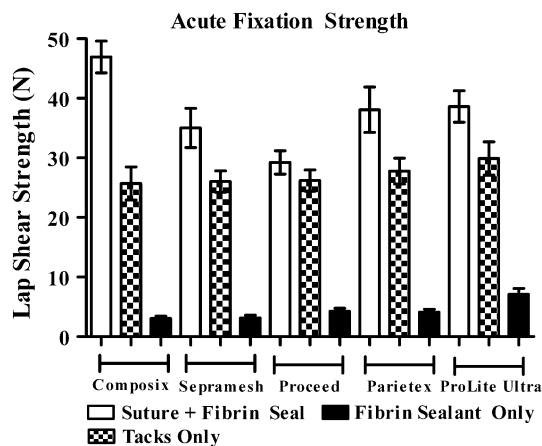


Fig. 4 Acute fixation strengths of suture + fibrin sealant, tacks only, and fibrin sealant for selected meshes

fixation with tacks alone did not differ any combination of meshes ($p > 0.05$ for all comparisons). The acute fixation strength achieved by FS alone did not differ for any combination of meshes ($p > 0.05$ for all comparisons).

Chronic mesh evaluation

Of the 75 chronic rabbits, 74 were kept alive for 8 weeks. A midline incisional hernia developed in one rabbit, unrelated to the implanted mesh, 35 days after implantation. The hernia sac was explored with the rabbit under general anesthesia using sterile technique with plans to repair the hernia. However, inspection of the two pieces of mesh secured with FS alone demonstrated clinical failure of both pieces of mesh (Proceed and Composix), and the

animal was sacrificed early. In fact, 48 of 50 meshes fixed with FS alone were found insufficiently affixed to the abdominal wall or affixed to the abdominal wall more than 2 cm away from the original implant location, which translated to a clinical failure rate of 96%.

Because most of the FS only samples were inadequately attached to the abdominal wall, it was not possible to measure mesh area, adhesion area, or adhesion tenacity in a standardized manner. Therefore, this group was excluded from statistical analysis. The suture + FS and tacks only samples showed no significant differences in mesh area ($p > 0.05$ for all comparisons). There were significant results regarding adhesion area, but they showed no general trend (Fig. 6). These findings included a significantly

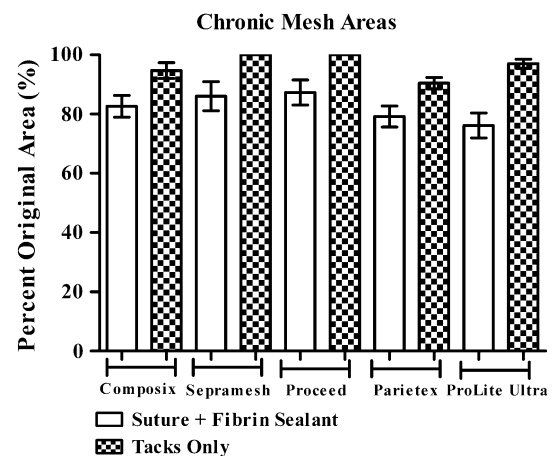


Fig. 5 Mesh area at explant for selected meshes attached with suture + fibrin sealant or tacks only

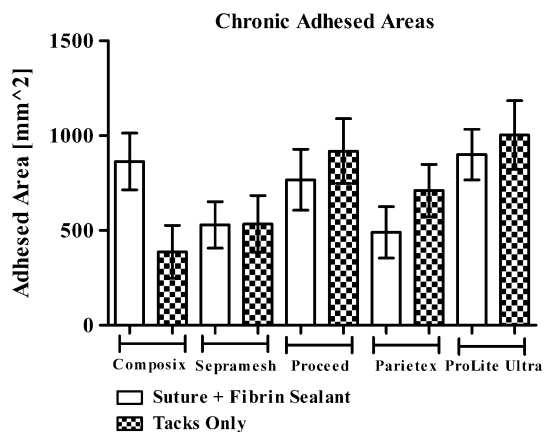


Fig. 6 Adhesed area at explant for selected meshes attached with suture + fibrin sealant or tacks only

greater adhesed area for ProLite Ultra secured with tacks only compared with Composix or Sepramesh ($p \leq 0.028$), for Proceed secured with tacks only compared with Composix ($p = 0.013$), and for Composix secured with suture + FS compared with tacks only ($p = 0.017$). There were no significant findings of adhesion tenacity (Fig. 7) for any mesh and fixation method combination ($p > 0.05$ for all comparisons).

Chronic fixation strength

The chronic fixation strengths for all mesh and fixation combinations are presented in Fig. 8. The chronic fixation strengths were significantly greater for all the meshes secured with either suture + FS or tacks alone compared with FS alone ($p \leq 0.0005$ for all comparisons). The chronic fixation strength was greater for suture + FS compared with tacks alone for Proceed and ProLite Ultra ($p \leq 0.013$). The chronic fixation strength with tacks alone

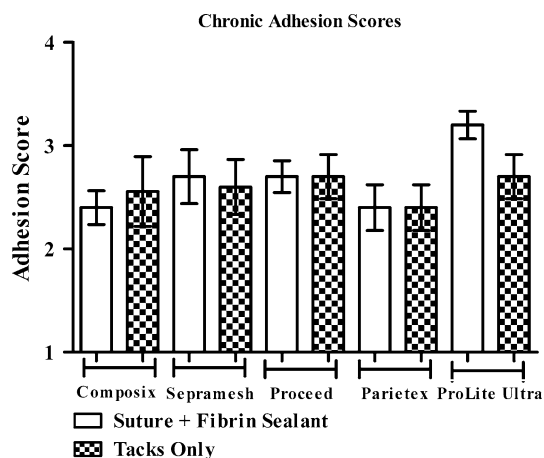


Fig. 7 Adhesion score at explant for selected meshes attached with suture + fibrin sealant or tacks only

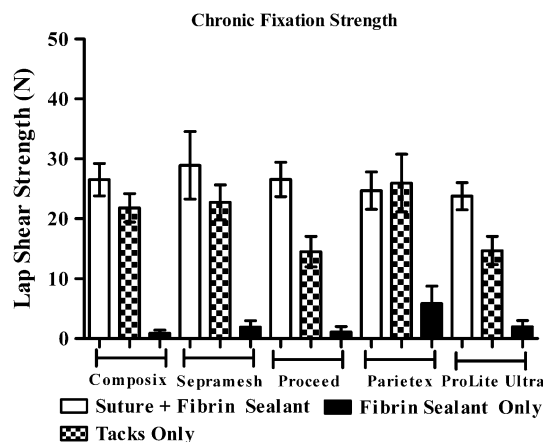


Fig. 8 Chronic fixation strengths of suture + fibrin sealant, tacks only, and fibrin sealant for selected meshes

was greater for Composix, Parietex Composite, and Sepramesh than for Proceed and Prolite Ultra ($p < 0.05$). The chronic fixation strength achieved by fixation with suture + FS for any combination of meshes ($p > 0.05$ for all comparisons) did not differ significantly from that achieved by FS alone for any combination of meshes ($p > 0.05$ for all comparisons).

Acute vs chronic fixation strength

The acute and chronic fixation strengths for all mesh and fixation combinations are compared in Fig. 9. The acute fixation strength was greater than the chronic fixation strength with suture + FS for Composix, Parietex Composite, and Prolite Ultra ($p \leq 0.0002$). The acute fixation strength was greater than the chronic fixation strength with tacks alone for Proceed and Prolite Ultra ($p \leq 0.0013$). No significant differences were observed between the acute and chronic fixation strengths achieved by fixation with FS alone for any mesh type ($p > 0.05$ for all comparisons).

Discussion

A general trend demonstrated by this study was that acute and chronic fixation strengths were greatest for suture + FS than for fixation with tacks only or FS only for selected nonabsorbable (Composix) and absorbable barrier-coated (Sepramesh, Proceed, and Parietex Composite Mesh) meshes. The results also demonstrated a general trend toward greater acute and chronic fixation strengths with tacks only than with FS only. The overall low acute fixation strengths achieved with FS only (ranging from 3.1 ± 0.3 N for Composix to 7.1 ± 1.0 N for ProLite Ultra) proved inadequate to maintain chronic fixation to the

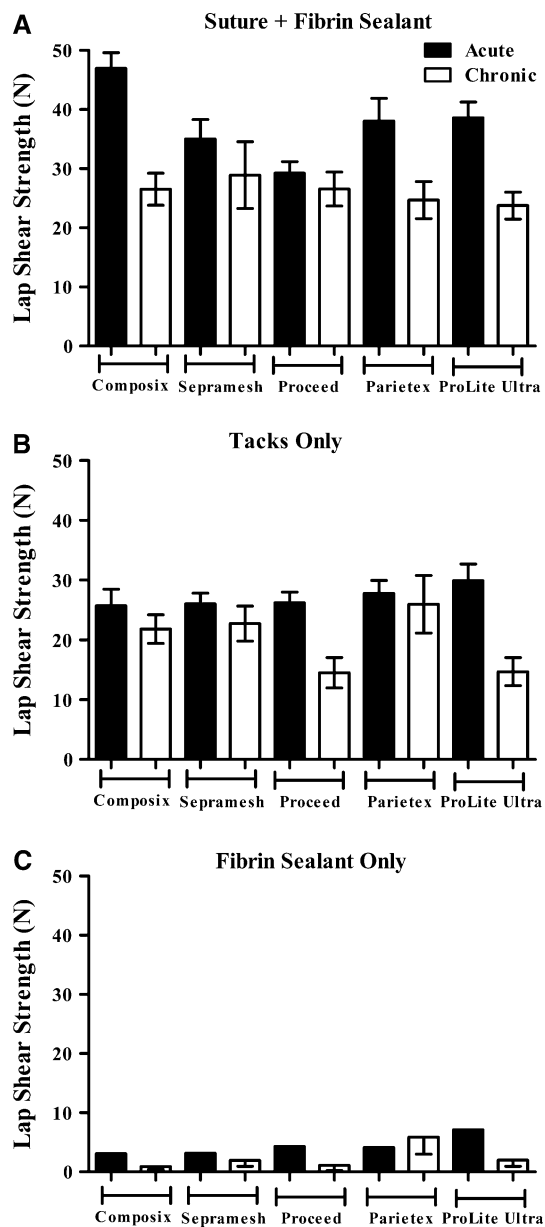


Fig. 9 Acute versus chronic fixation strengths for selected meshes using suture + fibrin sealant (a), tacks only (b), or fibrin sealant only (c) for fixation

peritoneum in this rabbit model, as evidenced by 48 chronic failures in 50 mesh fixations.

Lower recurrence rates have been shown with laparoscopic technique (4.3%) than with open technique (12.1%) in ventral hernia repair in humans [1]. These lower recurrence rates observed with LVHR assume traditional permanent mesh fixation via suture with or without tacks. The low acute fixation strength of FS alone leading to chronic mesh fixation failure in this rabbit model indicates the need for further evaluation before its use in humans as a sole means of mesh fixation in LVHR. However, suture + FS may be an alternative to fixation with suture + tacks. It

should be noted that FS currently is not FDA approved for mesh fixation. All applications of these products in laparoscopic TEP and TAPP repairs and LVHR are currently “off label” in the United States.

Current mesh fixation techniques are associated with chronic pain and intestinal adhesions, with rare reports of erosion, fistulization, or both [2–5, 16]. Research involving alternative fixation techniques in LVHR is ongoing to minimize or avoid these complications. Absorbable tacking/fixation devices and FS currently are used by some surgeons in an attempt to provide adequate acute fixation strength while decreasing the morbidity associated with permanent fixation materials.

To provide a successful repair, fixation constructs must provide adequate fixation strength until the mesh is adequately incorporated. However, in an intraperitoneal location, it is unclear whether mesh will be adequately incorporated to provide a permanent repair without permanent fixation devices due to the mesothelialized tissue surface. Studies to date have demonstrated inferior fixation strength of all products except 0-polypropylene suture [16, 17].

Before this series, only one study had evaluated the chronic location of mesh placed laparoscopically with FS fixation alone [18]. Importantly, two of the 18 pieces of mesh secured with FS alone were folded at the time the animals were sacrificed, which could have resulted in hernia recurrence in a hernia repair model. The study did not evaluate fixation strength because chronic incorporation strength was the only fixation parameter tested. At 30 days, the titanium tacks were removed from all samples, and mechanical testing was performed to determine strength of incorporation, not fixation strength, because all FS is degraded in 10–14 days [18].

The only human series of ventral hernias repaired laparoscopically using FS fixation was reported by Olmi et al [19]. Their series included 40 small to medium (2–7 cm) incisional and primary hernias repaired using Parietex Composite Mesh (Covidien) with 4–5 cm of overlap. They used an FS diluted to a thrombin concentration of 50 IU/ml as their solitary method of fixation. The FS was diluted to ensure adequate time for positioning of the prosthetic mesh before polymerization of the FS. During a mean follow-up period of 16 months (range, 3–24 months), no postoperative complications or recurrences were reported [19].

As evidenced by the multiple articles detailing the use of FS in laparoscopic TAPP and TEP repairs [8–13], FS is quickly gaining popularity as a sole method of mesh fixation. Some physicians are even referring to FS as the “gold standard method for mesh fixation” [10]. Arguably, adequate fixation for mesh in laparoscopic TAPP and TEP repairs does not equate with adequate fixation in LVHR. Despite only one human study to date using FS alone for

mesh fixation in LVHR, the participating physicians declared fixation with FS alone in LVHR as their “therapeutic option of choice” with small to medium incisional hernias [19].

Data cannot be extrapolated from laparoscopic TAPP and TEP repairs and applied to LVHR because the repair environments are different, including unique physiologic pressures. Proof that FS maintains mesh in place in the inguinal area, where it has been suggested that no fixation is needed [20–25], is not adequate evidence for performing LVHR without transfascial sutures or other fixation devices. And the reverse is equally true. Evidence from this study demonstrating that FS with a thrombin concentration of 4 IU/ml is not adequate as a sole fixation method in LVHR does not prove that FS alone would be inadequate for mesh fixation in laparoscopic TAPP and TEP repairs.

Evidence from the use of FS in lieu of fixation devices in laparoscopic TEP and TAPP suggests the possible benefit of decreased pain, both immediate and chronic, with the use of FS instead of tacks in LVHR. Efforts to minimize chronic postoperative pain attributed to fixation techniques in LVHR must be balanced with the need for adequate fixation strength to prevent hernia recurrences. As evidenced by this study, using a combination of suture and FS for mesh fixation in LVHR may provide adequate fixation while decreasing postoperative pain due to spiral titanium tacks or an alternative fixation device. Experimentation in a hernia repair model is warranted for further examination of suture and FS for mesh fixation in LVHR.

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