Evaluation of adhesion formation, mesh fixation strength, and hydroxyproline content after intraabdominal placement of polytetrafluoroethylene mesh secured using titanium spiral tacks, nitinol anchors, and polypropylene suture or polyglactin 910 suture

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Abstract

Background: The purpose of this study is to evaluate fixation methods for polytetrafluoroethylene (ePTFE) mesh with an *in vivo* model of laparoscopic ventral hernia repair.

Methods: In 40 New Zealand white rabbits, a 4×4-cm ePTFE mesh ($n = 80$, two per animal) was attached to an intact peritoneum with polyglactin 910 (PG 910) ($n = 20$) or polypropylene (PP) ($n = 20$) suture, titanium spiral tacks (TS) ($n = 20$), or nitinol anchors (NA) ($n = 20$). Mesh was harvested at 8 and 16 weeks for fixation strength testing, adhesion assessment, and collagen (hydroxyproline) content. Fixation strength on day 0 was determined with mesh attached to harvested abdominal wall. Statistical significance was determined as $p < 0.05$.

Results: There was no difference in fixation strength between PP (39.1 N) and PG 910 (40.0 N) sutures at time zero. At week 8, PP (25.7 N) was significantly stronger ($p < 0.05$) than PG 910 (11.4 N) suture, but not at week 16. The fixation strength of TS and NA (day 0, 15.4 vs 7.4 N; week 8, 17.5 vs 15.3 N; week 16, 19.1 vs 13.8 N) was not significantly different. Fixation with PP suture was significantly ($p < 0.05$) stronger than that with TS and NA at day 0 (39.1, 15.4, and 7.4 N, respectively) but not at weeks 8 or 16. The fixation strength of suture decreased significantly ($p < 0.05$) from day 0 to week 16 (PP: day 0 = 39.1 N, week 8 = 25.7 N, week 16 = 21.4 N; PG 910: day 0 = 40.0 N, week 8 = 11.4 N, week 16 = 12.8 N). The fixation strength of NA and TS did not change significantly (NA: day 0 = 7.4 N, week 8 = 15.3 N, week 16 = 13.8 N; TS: week 0 = 15.4 N, week 8 = 17.5 N, week 16 = 19.1 N). There were no differences in adhesion area based on fixation device used; however, there were more ($p < 0.05$) mesh samples using NA with adhesions compared to TS and adhesion tenacity was greater ($p < 0.05$) compared to that of TS, PP, and PG. Hydroxyproline content at weeks 8 and 16 was similar for all fixation devices.

Conclusions: The initial fixation strength for nonabsorbable suture is significantly greater than that of the metallic fixation devices, but after 8 weeks there is no difference. Laparoscopic ventral hernia repair without transabdominal suture fixation may be predisposed to acute failure. The metallic devices have similar fixation strength, although the incidence of adhesions and tenacity of adhesions appear to be greater with the nitinol anchors. Since these devices have similar fixation strengths and most likely provide adequate supplementation to transabdominal sutures for mesh fixation after laparoscopic ventral hernia repair, their use should be based on other factors, such as their propensity for adhesions, ease of application, and cost.

Laparoscopic ventral hernia repair has become a standard, reproducible method for repair of abdominal wall hernias [5]. Adequate fixation of a large piece of mesh to the anterior abdominal wall with sutures and/or fixation devices is required for a successful repair. The strength of the repair is initially dependent on the method of fixation, which may be supplanted by tissue ingrowth over

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time. Nonabsorbable suture supplemented by metallic fixation devices is the standard method for fixation, but circumferential, full-thickness abdominal wall sutures can contribute to significant acute postoperative pain as well as chronic pain syndromes requiring local anesthetic injection, intercostals nerve block, and/or possible removal of the offending stitch [2, 5]. In most circumstances, the use of tackers, staplers, and anchor fixation devices augments suture fixation strength in order to prevent hernia recurrence and limit the suture requirements. Some authors have described laparoscopic ventral hernia repair without transabdominal fixation sutures, but others believe that recurrence is often due to mesh migration prior to tissue ingrowth and sutureless fixation promotes failure [5]. Additionally, unlike sutures, fixation devices have the potential to dangle into the abdominal cavity, which can create an inflammatory reaction between the fixation device and the viscera, possibly resulting in intestinal adhesions with obstruction, erosion, and/or fistulization [2, 6–8]. This can ultimately increase patient morbidity and medical expenses.

The purpose of this study was to examine mesh fixation strength, intraperitoneal adhesion formation, and fibrocollagenous ingrowth (hydroxyproline content) after the intraabdominal placement of polytetrafluoroethylene (ePTFE) mesh (DualMesh, W.L. Gore & Associates, Flagstaff, AZ, USA) using 5-mm titanium spiral tacks (ProTack, United States Surgical Corporation, Norwalk, CT, USA), 5-mm nitinol anchors (EndoAnchor, Ethicon Endo-Surgery, Cincinnati, OH, USA), nonabsorbable polypropylene suture (Prolene, Ethicon, Somerville, NJ, USA), and absorbable polyglactin 910 suture (Vicryl, Ethicon) in a New Zealand white rabbit model. This study investigated the mesh–tissue fixation strength after the intraabdominal placement of ePTFE mesh using the four different fixation devices in order to determine which fixation method facilitates mesh attachment over a 16-week study period. A secondary aim was to determine which fixation device predisposes ePTFE mesh to adhesions.

Materials and methods

Approvals for this study was granted by the Institutional Animal Care and Use Committee at the Carolinas Medical Center. All animals were treated in compliance with the *Guide for the Care and Use of Laboratory Animals* [3]. The animals were acclimated to the vivarium for at least 2 weeks prior to mesh implantation.

Mesh implantation

Anesthesia was induced by an intramuscular injection of ketamine (20 mg/kg) and acetylpromazine (0.5 mg/kg given 30 min prior to the ketamine). Endotracheal intubation was performed and intraoperative anesthesia was maintained with 3.0% isoflurane inhalation. After a midline incision, a 4 × 4-cm piece of ePTFE mesh ($n = 80$, two per animal) was attached to an intact peritoneum with 2–0 polyglactin 910 (PG 910) suture ($n = 20$), 2–0 polypropylene (PP) suture ($n = 20$), titanium spiral tacks (TS) ($n = 20$), or nitinol anchors (NA) ($n = 20$) in 40 New Zealand white rabbits. Two pieces of mesh were placed in each animal on opposite sides of the midline (Fig. 1 and 2). For each 4 × 4-cm piece of ePTFE mesh, 14 fixation devices were used to secure the

Fig. 1. Fixation of ePTFE mesh against the peritoneum with a nitinol anchor.
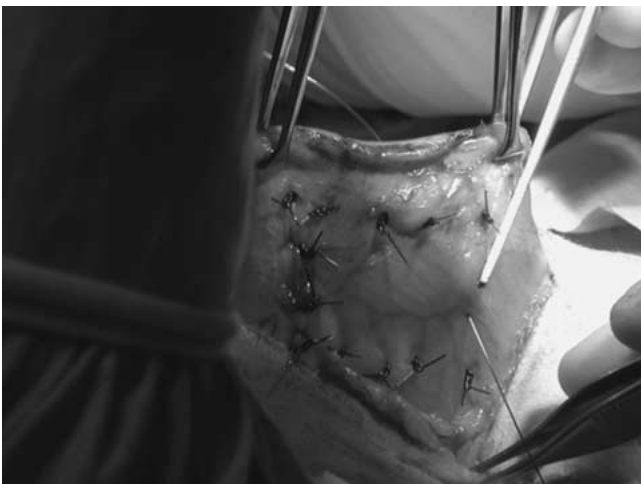
Fig. 2. Transabdominal fixation sutures (polypropylene) with knots in the subcutaneous tissue.

biomaterial. The sutures were placed transabdominally with the knots residing in the subcutaneous tissue. Which fixation device was used for each site was determined by randomization using a computer-generated random number scheme. After mesh placement, the abdominal wall midline fascia was closed with 3–0 absorbable suture and the subcutaneous tissue was closed with a 3–0 absorbable, subcuticular suture.

Necropy and mesh explantation

The animals were killed by pentobarbitol overdose (> 300 mg/kg, i.v.) after ketamine (35 mg/kg, i.m.) sedation. Randomization using a computer-generated random number scheme determined which animal was killed based on the fixation device placed. Twenty animals were killed at 8 weeks (mesh, $n = 40$, 10 per fixation device) and 16 weeks (mesh, $n = 40$, 10 per fixation device). The abdominal wall, mesh, and any adhesions were harvested en bloc. The specimen was evaluated for adhesion formation. After adhesion scoring, the mesh samples were divided into two 2 × 4-cm pieces for fixation strength and collagen (hydroxyproline) content determination, respectively.

Adhesion assessment

Adhesions were evaluated for quantity (No. of pieces of mesh with adhesions), tenacity, and area (mean %). An adhesion scale (1–4) was

Table 1. Adhesion scale[a]

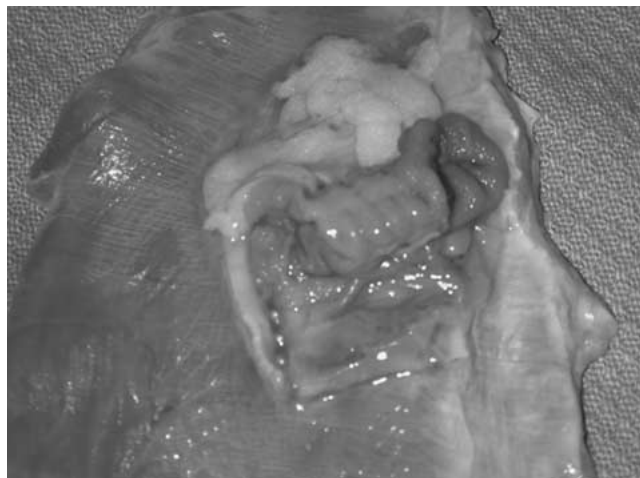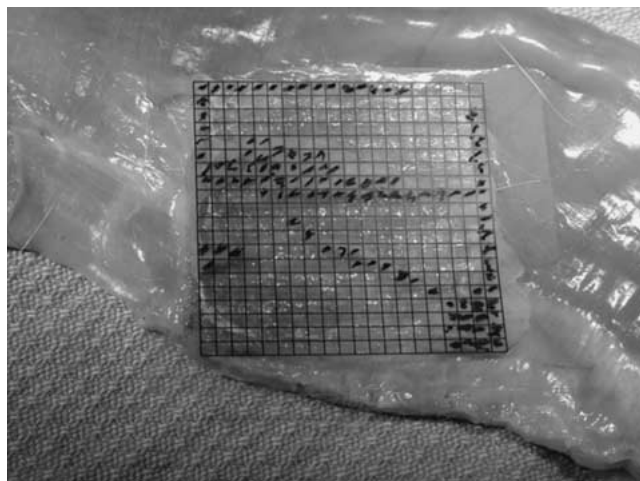| Type of adhesions	Score
No adhesions	1
Filmy adhesions, easily broken manually	2
Dense adhesions, requiring blunt dissection to separate viscera from mesh	3
Very dense adhesions, viscera matted to mesh surface, requiring sharp dissection to separate viscera from mesh	4

[a] From Garrard et al. [4]

Fig. 3. Adhesion scale score = 2.

Fig. 4. Adhesion scale score = 3.

Fig. 5. Adhesion scale score = 4.

Fig. 6. Adhesion area assessment (%) using a transparent grid.

used to assess adhesion tenacity (Table 1) [4]. Examples of tenacity scores of 2 (Fig. 3), 3 (Fig. 4), and 4 (Fig. 5) are provided. Adhesion area was determined using a transparent grid overlay technique (Fig. 6). A 4 × 4-cm transparency with an imprinted grid of 2 × 2-mm squares was placed over the mesh and intact adhesions. The number of squares with adhesions were counted. The total of these squares over 100 (the total number of squares) provided a percentage of adhesions to the ePTFE mesh.

Fixation strength testing

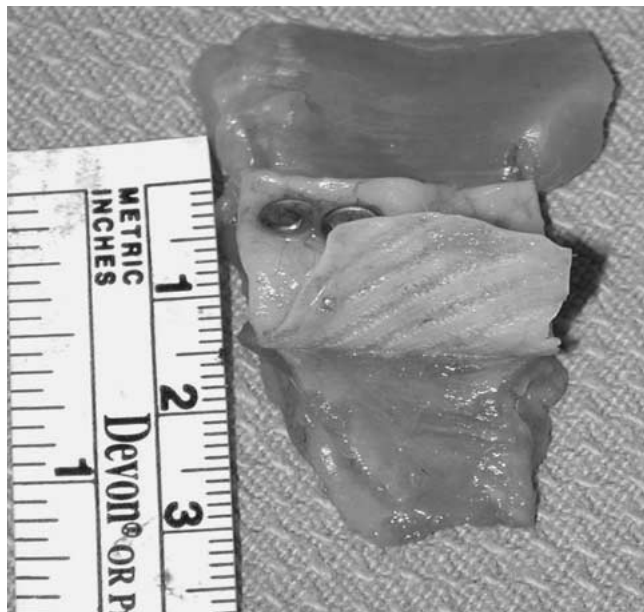After the abdominal wall/mesh sample was divided into two equal samples (Fig. 7), one sample immediately underwent fixation strength testing using a tensiometer (MN44, Instron, Canton, MA, USA). Two pneumatic grips were attached to opposite ends of the sample—one to the free, lateral abdominal wall without mesh and the other to the middle edge of the mesh (Fig. 8). Failure of fixation was determined after a tangential pull with a displacement rate of 25 mm/sec. Fixation strength at on day 0 was determined with mesh attached to harvested abdominal wall.

Collagen (hydroxyproline) content

The abdominal wall/mesh sample was divided into two equal samples after adhesion assessment, as previously described. The second sample was utilized to calculate hydroxyproline content. Hydroxyproline content was determined from the harvested samples after digesting the samples in papain and subsequent hydrolysis with 6 N hydrochloric acid. Spectrophotometric absorbance (557 nm) was compared to known concentrations to determine the concentration of hydroxyproline in the mesh samples.

Statistical analysis

Standard statistical methods were utilized. To compare the mean fixation strength and hydroxyproline content among the four groups, one-way analysis of variance (ANOVA) was performed. The severity

Fig. 7. Harvested abdominal wall/mesh sample divided for fixation strength testing.

Fig. 8. Fixation strength testing of mesh/abdominal wall sample using an Instron MN44 tensiometer.

of adhesions (tenacity) was compared with the Kruskal-Wallis test. The percentage of mesh covered with adhesions was compared using ANOVA. The data were analyzed using the SAS software. A p value < 0.05 was considered statistically significant.

Table 2. Analysis of adhesion scoring for combined results of 8-week and 16-week ePTFE mesh samples

	Polypropylene	Polyglactin 910	Titanium spiral tacks	Nitinol anchors
Mesh with adhesions (%)	30	30	35	70*
Mean adhesion score	1.65	1.55	1.60	2.75
Mean adhesion area (cm^2)	0.77	0.75	0.54	2.56*

*$p < 0.05$

Table 3. Mean tissue–mesh fixation strength (N)

Week	Polypropylene	Polyglactin 910	Titanium spiral tacks	Nitinol anchors
0	39.1 N*	40.0 N*	15.4 N*	7.4 N*
8	25.7 N*	11.4 N*	17.5 N	15.3 N
16	21.4 N*	12.8 N*	19.1 N	13.8 N

*$p < 0.05$

Results

There were no statistical differences in adhesion area based on the fixation device used (Table 2). However, ePTFE mesh samples secured with nitinol anchors were more likely ($p < 0.05$) to develop adhesions and the adhesion tenacity score was greater ($p < 0.05$) than for samples fixed with titanium spiral tacks, polypropylene suture, and PG 910 suture.

The results of fixation strength testing for the samples are summarized in Table 3. There was no difference in fixation strength between polypropylene (39.1 N) and PG 910 (40.0 N) sutures at time zero. At week 8, polypropylene (25.7 N) was significantly stronger ($p < 0.05$) than PG 910 (11.4 N) suture, but not at week 16. The fixation strength of titanium spiral tacks and nitinol anchors (day 0, 15.4 vs 7.4 N; week 8, 17.5 vs 15.3 N; week 16, 19.1 vs 13.8 N) was not significantly different. Fixation with polypropylene suture was significantly ($p < 0.05$) stronger than titanium spiral tacks and nitinol anchors at day 0 (39.1, 15.4, and 7.4 N, respectively) but not at weeks 8 and 16. The fixation strength of suture decreased significantly from day 0 to week 16 (polypropylene: day 0 = 39.1 N, week 8 = 25.7 N, week 16 = 21.4 N; PG 910: day 0 = 40.0 N, week 8 = 11.4 N, week 16 = 12.8 N). The fixation strength of nitinol anchors and titanium spiral tacks did not change significantly (NA: day 0 = 7.4 N, week 8 = 15.3 N, week 16 = 13.8 N; TS: week 0 = 15.4 N, week 8 = 17.5 N, week 16 = 19.1 N).

Week 8 and week 16 hydroxyproline content (microgram/ml) was not different based on fixation device or time of explant/testing. The results of hydroxyprolene content calculation are summarized in Table 4.

Discussion

Fixation devices are critical to the success of laparoscopic ventral hernia repair, but studies evaluating the

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Table 4. Mean hydroxyproline content (mg/ml)

Week	Polypropylene	Polyglactin 910	Titanium spiral tacks	Nitinol anchors
8	9.9	7.5	12.2	5.9
16	3.9	3.9	4.6	5.7

various techniques have been limited. Ingrowth and mesh stabilization should be maximized while minimizing the complications associated with these devices, such as adhesions and their sequelae and acute or chronic pain syndromes. This study demonstrated a significant loss of strength by the absorbable, PG 910 sutures at 8 weeks, suggesting that their use alone would not be adequate compare to nonabsorbable, polypropylene suture. Additionally, the initial (time zero) fixation strength for the absorbable and nonabsorbable suture material was significantly greater than that of the titanium spiral tacks and nitinol anchors, suggesting that the metallic devices alone do not provide adequate acute fixation prior to tissue ingrowth and mesh stablization. Van't Riet et al. [9] reported similar results for acute fixation strength testing comparing helical titanium coils and transabdominal sutures. Nevertheless, week 8 and week 16 fixation strength with the titanium spiral tacks and nitinol anchors was equivalent to that of nonabsorbable suture in our study. The minimum time period during which acute fixation strength is superior for nonabsorbable suture compared to the metallic fixation devices has not been determined. In a porcine ventral hernia model with repairs performed laparoscopically, Winslow et al. [10] demonstrated equivalent mesh–tissue tensile strength for Bard Composix E/X Mesh (Davol, Cranston, RI, USA) fixed with spiral tacks and prolene suture 4 weeks after implantation.

A potential limitation of this study is that the New Zealand white rabbit abdominal wall is relatively thin and may not be representative of the thicker human abdominal wall. The majority of the metallic fixation devices penetrated multiple fascial layers of the abdominal wall in our rabbit model. In this model, tensile strength may be falsely elevated for the metallic fixation device groups compared to a similar situation in humans when the titanium spiral tacks and nitinol anchors do not penetrate the abdominal wall fascia with any consistency. One of the concerns of performing laparoscopic ventral hernia repair without the use of transabdominal fixation sutures in humans is that the acute fixation strength for titanium spiral tacks and nitinol may be suboptimal, as demonstrated in our animal model. It is likely that a combination of transabdominal sutures and metallic fixation devices is optimal in order to maximize initial mesh fixation strength (transabdominal fixation sutures) and to limit suture-related complications, such as acute and chronic pain syndromes. Nevertheless, laparoscopic ventral hernia repair has been performed without transabdominal fixation sutures with good short-term outcomes [1]. A prospective, randomized study comparing laparoscopic

ventral hernia repair with and without transabdominal fixation sutures has not been completed. Another potential pitfall is that the study was not completed in a ventral hernia model. Abdominal wall physiology (compliance, distribution of force, etc.) is distorted with fascial defects and may have altered 8-week and 16-week fixation strength testing.

Adhesions occurred more commonly with the nitinol anchor than with the other fixation methods, which is most likely related to its larger intraabdominal profile. Sutures and the titanium spiral tacks are easily positioned flush against the mesh and/or abdominal wall, whereas a portion of the anchors extends a few millimeters into the abdomen, making them more likely to contact omentum and intraabdominal viscera. Even so, a higher adhesion score for sutured (prolene) mesh compared to mesh fixed with spiral tacks has been demonstrated at 4 weeks in a porcine model of laparoscopic ventral hernia repair [10]. A composite mesh of ePTFE bonded to polypropylene was used in this study and may have contributed to the differences noted. In addition to the increased total number of pieces of mesh with adhesions in our series after fixation with nitinol anchors, the adhesion tenacity score was significantly greater as well. Despite these findings, the total surface area of mesh covered by adhesions was equivalent for ePTFE fixed with titanium spiral tacks, nitinol anchors, absorbable and nonabsorbable suture.

Collagen ingrowth is one of the primary aspects of tissue healing, and hydroxyproline content was used as a marker of tissue ingrowth in this study. Hydroxyproline content determination on the mesh revealed no differences based on the type of device used to secure the mesh to the abdominal wall. This suggests that strength differences between the devices are related to the devices and not due to an alteration in tissue reaction that promoted or inhibited tissue ingrowth. Inflammatory response at the site of the fixation devices was not evaluated in this study.

Controversy regarding the most appropriate technique for mesh fixation will most assuredly continue. This study supports the use of transabdominal fixation with sutures in laparoscopic ventral hernia repair in order to allow early fixation before adequate tissue ingrowth. Absorbable suture may be an option in the future. Additional studies must be performed to ensure adequate fixation strength when absorbable suture is combined with metallic fixation devices. Both nitinol anchors and titanium spiral tacks provide adequate supplemental strength to be used in laparoscopic ventral hernia repair, although the incidence of adhesions and tenacity of adhesions appear to be greater with the nitinol anchors. Since these devices have similar fixation strength and total surface area of mesh covered with adhesions at each time period, their use should be based on other factors, such as their propensity for adhesions or tenacity of adhesions, ease of application, and cost.

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