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Histologic and biomechanical evaluation of a novel macroporous polytetrafluoroethylene knit mesh compared to lightweight and heavyweight polypropylene mesh in a porcine model of ventral incisional hernia repair

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Abstract

Purpose—To evaluate the biocompatibility of heavyweight polypropylene (HWPP), lightweight polypropylene (LWPP), and monofilament knit polytetrafluoroethylene (mkPTFE) mesh by comparing biomechanics and histologic response at 1, 3, and 5 months in a porcine model of incisional hernia repair.

Methods—Bilateral full-thickness abdominal wall defects measuring 4 cm in length were created in 27 Yucatan minipigs. Twenty-one days after hernia creation, animals underwent bilateral preperitoneal ventral hernia repair with 8 × 10 cm pieces of mesh. Repairs were randomized to Bard®Mesh (HWPP, Bard/Davol, <http://www.davol.com>), ULTRAPRO® (LWPP, Ethicon, <http://www.ethicon.com>), and GORE®INFINIT Mesh (mkPTFE, Gore & Associates, <http://www.gore.com>). Nine animals were sacrificed at each timepoint (1, 3, and 5 months). At harvest, a 3 × 4 cm sample of mesh and incorporated tissue was taken from the center of the implant site and subjected to uniaxial tensile testing at a rate of 0.42 mm/s. The maximum force (N) and tensile strength (N/cm) were measured with a tensiometer, and stiffness (N/mm) was calculated from the slope of the force-versus-displacement curve. Adjacent sections of tissue were stained with hematoxylin and eosin (H&E) and analyzed for inflammation, fibrosis, and tissue ingrowth. Data are reported as mean ± SEM. Statistical significance ($P < 0.05$) was determined using a two-way ANOVA and Bonferroni post-test.

Results—No significant difference in maximum force was detected between meshes at any of the time points ($P > 0.05$ for all comparisons). However, for each mesh type, the maximum strength at 5 months was significantly lower than that at 1 month ($P < 0.05$). No significant difference in stiffness was detected between the mesh types or between timepoints ($P > 0.05$ for all comparisons). No significant differences with regard to inflammation, fibrosis, or tissue ingrowth were detected between mesh types at any time point ($P > 0.09$ for all comparisons). However, over time, inflammation decreased significantly for all mesh types ($P < 0.001$) and tissue ingrowth reached a slight peak between 1 and 3 months ($P = 0.001$) but did not significantly change thereafter ($P > 0.09$).

Conclusions—The maximum tensile strength of mesh in the abdominal wall decreased over time for HWPP, LWPP, and mkPTFE mesh materials alike. This trend may actually reflect inability to adequately grip specimens at later time points rather than any mesh-specific trend. Histologically, inflammation decreased with time ($P = 0.000$), and tissue ingrowth increased ($P = 0.019$) for all meshes. No specific trends were observed between the polypropylene meshes and the monofilament knit PTFE, suggesting that this novel construction may be a suitable alternative to existing polypropylene meshes.

Keywords

Polypropylene; Knit polytetrafluoroethylene; Ventral hernia repair; Biomechanical evaluation; Histologic response; Tissue remodeling

Introduction

Incisional hernias are a recurring problem in abdominal surgery and occur in as many as 20% of patients who have undergone laparotomy [1, 2]. Primary tissue repair of even small (less than 10 cm²) hernia defects has been associated with recurrence rates of between 40 and 60% [3]. As such, this approach has been largely abandoned in favor of repair with prosthetic mesh materials. However, the challenge of mimicking abdominal wall biomechanics while minimizing the risk for wound complications inherent with implantation of foreign materials has resulted in a veritable explosion of candidate products as companies compete to produce the best prosthetic mesh. In addition to polypropylene, polyester and expanded polytetrafluoroethylene (ePTFE) have recently become popular materials. Additionally, meshes with a seemingly endless array of partially absorbable fibers, multi-filament versus monofilament construction, barrier-coated surfaces, and varying pore size make it increasingly difficult to determine which mesh is best for a particular application. In the quest to characterize physiologic host responses to hernia meshes, we have chosen to compare biomechanical and histologic profiles of the following three meshes: Bard®Mesh

(HWPP, Bard/Davol, <http://www.davol.com>), ULTRAPRO® (LWPP, Ethicon, <http://www.ethicon.com>), and a novel, knitted polytetra-fluoroethylene mesh, GORE®INFINIT Mesh (mkPTFE, Gore & Associates, <http://www.gore.com>).

Materials and methods

This study was conducted under a protocol approved by the Washington University School of Medicine Animal Studies Committee. Twenty-seven ($n = 27$) female Yucatan minipigs were utilized. Animals were housed, fed, and handled according to the Guide for the Care and Use of Laboratory Animals [4] and standard protocols on file with the Washington University Division of Comparative Medicine.

Surgical preparation and creation of incisional hernia defects

Operative preparation and procedure for creating incisional hernias in minipigs has been previously described by our group [5]. Briefly, animals were fasted the night before surgery. Anesthesia was induced with an intramuscular injection of 4 mg/kg telazol (Wyeth, Madison, NJ), 2 mg/kg ketamine, and 2 mg/kg xylazine (Bayer, Leverkusen, Germany). Intraoperative general endotracheal anesthesia was maintained with 1–3.0% isoflurane, and intravenous saline given as needed during the operative procedure. Prior to incision, cefazolin (35 mg/kg) was administered intramuscularly, and the skin was prepped with standard povidone-iodine scrub solution. Rigorous surgical scrub and sterile technique was utilized at all times.

Two, 4-cm longitudinal incisions were made bilaterally 8 cm from the midline and carried out through skin, subcutaneous fat, fascia, and aponeurotic muscle layers. The peritoneum was left intact. The deep dermal layer was reapproximated with interrupted 3-0 PDS leaving the underlying layers of muscle and fascia open, and the skin was closed with interrupted subcuticular 4-0 PDS suture. Local analgesia was obtained with 0.5% bupivacaine injected at each site. Cyanoacrylate-based dermal glue was applied to seal out fluid and fecal contamination. Animals were monitored during recovery and allowed to resume their regular diet within 4 h. Postoperatively, analgesia was provided by administration of buprenorphine (0.02–0.05 mg/kg, IM) and Carprofen (2.2 mg/kg, PO) on a scheduled and as-needed basis until 36 h. Postoperative antibiotic prophylaxis was provided with 20–25 mg/kg oral cefazolin every 12 h for a total of 5 days.

Mesh repair of ventral hernia defects

Abdominal wall defects were left untreated for 21 days to allow for hernia development. At the time of repair, two 8 × 10 cm meshes (randomized preoperatively) were implanted in each animal. Bard®Mesh (bare polypropylene mesh with small pores) was selected as the HWPP, ULTRAPRO® (large-pore knit mesh constructed of polypropylene and poliglecaprone 25 absorbable filaments) was chosen as the LWPP, and were compared to GORE®INFINIT (a novel large-pore knit monofilament polytetrafluoroethylene). All meshes were used directly out of the original packaging and none were re-sterilized on site. Previously created wounds were opened to, but not through, the underlying peritoneum, and the retromuscular space was developed with blunt dissection. Meshes were positioned in the preperitoneal space with the long edge (10 cm) running axially and the short edge (8 cm) running transversely. The mesh was secured with eight transfascial 0-polypropylene sutures placed through stab incisions in the skin such that each suture came through the mesh approximately 3–4 cm apart and at least 1 cm from the edge. This repair allowed the piece of mesh to be centered under the hernia defect with 2–3 cm of overlap of the mesh onto the fascia circum-ferentially (Fig. 1). After confirming correct placement, sutures were tied down with knots buried in the subcutaneous tissue. The attenuated aponeurotic hernia sac

was reapproximated over the mesh with interrupted #0 PDS suture to provide an extra barrier against infection, and the deep dermal layer and skin were closed as previously described. Local analgesia was provided at both the main incision and transfascial suture sites 0.5% bupivacaine. Cyanoacrylate-based glue was applied as previously described and postoperative recovery, care, and treatments were conducted as described above.

Mesh retrieval and biomechanical testing

Mesh samples were allowed to remain in vivo for 1, 3, or 5 months, at which time the animals were sedated with intramuscular telazol (4 mg/kg), xylazine (2 mg/kg), and ketamine (2 mg/kg) and euthanized with an overdose of IV pentobarbital (100 mg/kg) or potassium chloride (>100 mg/kg IV). The abdominal cavity was opened longitudinally along the midline, allowing gross in situ inspection of the mesh samples. The abdominal wall was harvested en bloc from the animal and trimmed to one muscle layer to allow specimens to fit into grips for tensile testing. The peritoneum, transversus abdominis muscle layer, and incorporated mesh was contained in all specimens. Due to the amount of preperitoneal fat in the porcine abdominal wall, it was difficult to define the edges of the mesh samples consistently. Thus, to ensure consistency in sampling, specimens for tensile testing and histologic analysis were both cut from the center of each implant site. This ensured that mesh was equally included in all samples: from the 4 × 4 cm specimens of mesh-abdominal wall composite, a 1 × 4 cm strip was removed for histologic testing. The remaining 3 × 4 cm piece was used for biomechanical testing using an Instron Series 5542 Universal Testing System (Instron, Norwood, MA). Each specimen was oriented in the vertical direction and tested to failure at a rate of 0.42 mm/s (1 inch/min) (Fig. 2). The maximum load sustained by the specimen was recorded in Newtons (N), and the tensile strength per unit width was calculated by dividing the maximum load sustained by the material by the width of the specimen (N/cm). The approximate stiffness (N/mm) was calculated from the slope of the force-vs-displacement curve in the linear region of the curve. Six specimens were tested for each type of implanted mesh ($n = 6$) at each time point.

Histologic evaluation of samples

For histologic analysis, a 1 × 1 cm piece of mesh-tissue composite was embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for analysis under light microscopy at 40, 100, and 200× magnification. Five to ten non-overlapping fields per specimen were evaluated at 100× magnification and graded by a veterinary pathologist according to a scale adapted from the American Society for Testing and Materials (ASTM) guidelines (Tables 1, 2) [6]. Higher scores correlate with higher levels of inflammation and fibrosis or a greater degree of tissue ingrowth. Overall response scores reflect the collective average of the inflammatory and fibrosis scores.

Statistical analysis

Data was analyzed with Systat 12.0 software (Chicago, IL) using a two-way ANOVA, followed by a Bonferroni post-test to determine whether significant differences were observed. A P value of <0.05 was considered statistically significant. Data are presented as the mean ± SEM.

Results

Biomechanical results

Maximum forces sustained at 1, 3, and 5 months post-implantation were not significantly different from each other ($P > 0.05$ for all comparisons). However, each mesh individually exhibited significantly decreasing maximum forces by the 5 month time point (Fig. 3a). For

example, HWPP (Bard®Mesh) decreased from 59.3 ± 7.2 N at 1 month to 47.6 ± 6.7 and 36.0 ± 5.5 N at 3 and 5 months, respectively. The mkPTFE mesh (GORE®INFINIT Mesh) sustained 67.1 ± 6.8 N at 1 month and similarly decreased to 49.9 ± 7.3 and 40.8 ± 5.1 N over time, while LWPP mesh (ULTRAPRO®) trended down from 61.5 ± 5.2 – 46.8 ± 5.1 N and 37.8 ± 8.6 N, respectively, by 5 months. Tensile strength (N/cm) followed a similar trend: no significant differences were observed among meshes at each time point ($P > 0.05$). However, for each mesh, the strength at 5 months was significantly lower than 1 month $P < 0.05$ (Fig. 3b). Measurement of stiffness (N/mm) revealed no differences with respect to time or mesh type $P > 0.05$ (Fig. 3c).

Macroscopic results

There appeared to be some differences in the gross appearance of the meshes upon harvest. The HWPP samples appeared to undergo some distortion, causing the mesh edges to roll, whereas the LWPP and mkPTFE meshes appeared less prone to buckling (Fig. 4). It should also be noted that no recurrent hernias were observed in any of the animals.

Histologic results

Histologic analysis revealed no significant difference in inflammation, fibrosis, tissue ingrowth, or overall response scores (Fig. 5) at any of the three time points between the HWPP, LWPP, and mkPTFE meshes ($P = 0.17$). However, for each material over time, scores were significantly decreased for inflammation ($P = 0.000$), increased for tissue ingrowth ($P = 0.019$), and decreased for overall response scores ($P = 0.000$). There was no significant trend with respect to fibrosis ($P = 0.0761$). Examples of photomicrographs taken at 100× magnification are shown in Fig. 6.

The HWPP specimens exhibited macrophages, lymphocytes, multinucleated giant cells, and some focal hemorrhage at 1 month. Similarly, macrophages, lymphocytes, and minimal focal hemorrhage were also observed at 3 months with the addition of evidence of fibrosis near the mesh fibers. The LWPP specimens exhibited neutrophils, multinucleated giant cells, lymphocytes, macrophages, and some evidence of fibrosis at 1 month and evidence of lymphocytes and macrophages at 3 months. The mkPTFE specimens exhibited neutrophils, lymphocytes, macrophages, and multinucleated giant cells at 1 month, followed by relatively few inflammatory cells, and minimal focal hemorrhage at 3 months. By 5 months, all three meshes exhibited very few neutrophils and plasma cells, a few lymphocytes, and some macrophages.

Discussion

The hernia mesh revolution began in the 1940s with the use of prosthetics made from metals such as steel or tantalum [7]. Polypropylene knit mesh (Marlex) was introduced by Dr. Francis Usher in the late 1950s and offered superior pliability as well as better resistance to corrosion and migration compared to its metallic counterparts [8]. Since then, hernia meshes have evolved to include generations of lighter-weight meshes in addition to the original “heavyweight” polypropylene. Bellon et al. have described three categories for modern polypropylene meshes based on density: lightweight (<35 g/m²), mid-weight (35 – 80 g/m²), and heavyweight (>80 g/m²) [6, 7]. Several authors agree that lightweight knit polypropylene meshes offer several advantages including increased abdominal wall compliance, reduced inflammatory reactivity, and subsequent improvement in tissue incorporation. However, it has been well documented that polypropylene can be associated with intestinal adhesions and fistulization, causing manufacturers to continue to explore other materials [9–12].

Use of modern prosthetic meshes in combination with a laparoscopic approach to repair incisional hernias has reduced recurrence rates to between 0 and 10% [7, 13]. While polypropylene remains one of the most popular materials for incisional hernia repair due to its relatively low production cost, favorable handling characteristics, and ability to become well-incorporated into the abdominal wall [14], it nevertheless incites a foreign body reaction at the implant site; a phenomenon some authors believe leads to mesh shrinkage and subsequent loss of abdominal wall compliance [11]. Furthermore, there is evidence that traditional heavyweight polypropylene may be over-engineered since it possess a tensile strength far in excess of what is actually required; Klinge et al. [15] have published rupture forces for heavyweight polypropylene in excess of 100 N/cm, while normal physiologic forces acting on the abdominal wall range from 4 to 16 N/cm for small defects in which the defect can be closed. Meshes must provide even more strength, on the order of 32 N/cm in the case of large defects that cannot be closed. Furthermore, at 16 N/cm, the abdominal wall distends $25 \pm 7\%$, while heavyweight polypropylene meshes display a value of only 4–16% strain (i.e., stretch). This excessive rigidity is likely the culprit for postoperative complaints of discomfort, especially at the edges of the mesh [16]. Lighter-weight meshes, on the other hand, display elasticities closer to the physiologic range [17]. Meshes with reduced polypropylene content and increased pore size also demonstrate improved integration at the implant site in humans [17, 18]. Further problems with bare polypropylene in the intra-abdominal space are related to adhesion formation when placed in direct contact with abdominal viscera, which can lead to chronic pain, intestinal obstruction, infertility, fistulization, and chronic infection perpetuating a potential cycle of mesh removal and re-repair [7, 19–23].

Other examples of synthetic materials currently in use include knit multifilament polyester and microporous expanded polytetrafluoroethylene (ePTFE). ePTFE has been shown to reduce intra-abdominal adhesions, but it is not without limitations; meshes constructed with a sheetlike layer of ePTFE as an anti-adhesive barrier, while effective, can be associated with decreased incorporation into the abdominal wall, which can increase the risk for postoperative seroma and/or wound infection [14]. Additionally, once ePTFE mesh becomes contaminated, it must be removed. However, this material continues to be used widely in elective cases due to its proven long-term clinical performance; Heniford et al. [24] reported a 4.7% recurrence rate at a mean follow up of 20 months, and Wassenaar et al. [25] reported a recurrence rate of 1.8% at a mean follow up of 31 months for large hernia defects.

The current study was designed to evaluate and directly compare the biocompatibility and biomechanics of a novel mkPTFE mesh (GORE®INFINIT) against two existing knit-construction polypropylene meshes. This novel macroporous PTFE mesh design (GORE®INFINIT) should not be confused with the existing microporous ePTFE mesh design (DUALMESH®). The large-pore knit structure of GORE®INFINIT is intended to provide less surface area in order to encourage tissue ingrowth, and the monofilament fiber design is intended to reduce the risk of infection by reducing potential bacterial colonization sites. In contrast with the microporous ePTFE mesh (DUALMESH), this novel macroporous PTFE material should not be utilized for intraperitoneal placement. Adhesion or ingrowth of abdominal viscera through this macroporous mesh could lead to erosion, fistula, obstruction, or chronic pain as observed with many other macroporous mesh designs. [7, 19–23] Thus, the aim of the current study was to determine whether this novel mesh exhibits similar tissue ingrowth characteristics compared to existing macroporous polypropylene materials in a porcine model of incisional hernia repair.

Over a period of 5 months, maximum force and tensile strength decreased in all three meshes (HWPP, mkPTFE, and LWPP), with no significant change in stiffness. All three materials maintained a tensile strength within the 4–16 N/cm range for small hernia defects

suggested by Klosterhalfen and colleagues [26]. Additionally, none of the materials were associated with hernia recurrence or disruption anywhere at the implant site. During the course of the study, we discovered that, as the samples remained in vivo for longer periods of time, they accumulated increasingly larger preperitoneal fat deposits as the animals grew and gained weight. This made it increasingly difficult to test specimens to the point of failure because samples slipped out of the machine grips before reaching ultimate failure. As such, we are unable to report on the ultimate tensile strength of the mesh-tissue composite at the repair site. Fortunately, however, the maximum load measured for the majority of the specimens occurred near the physiologic range of approximately 50–90 N before the specimens slipped from the grips. Therefore, the ultimate tensile strength of the mesh-tissue composite would likely have been above the physiologic range if it had been measured to failure of the specimen. To eliminate confounding variables, we based all stiffness measurements and calculations on the linear portion of the force-vs-displacement curve for each test run. It should also be noted that, except for ULTRAPRO[®], mesh implants were oriented randomly with respect to the *X* and *Y* direction of the knit, to mimic clinical application. Specimens were thus tested in random orientations with regard to mesh knit direction, possibly affecting strength measurements.

Overall histologic scores reflected progressive tissue ingrowth and decreasing levels of inflammation and fibrosis for all meshes over a period of 5 months, consistent with a normal course of healing and wound maturation. Although our histologic analysis did not detect significant differences in foreign body reaction at the cellular level between LWPP and HWPP, there appeared to be some differences in the gross appearance of the meshes upon harvest. The HWPP samples appeared to undergo some distortion, causing the mesh edges to roll, whereas the LWPP and mkPTFE meshes seemed less prone to buckling (Fig. 4). These results are consistent with what other authors have observed in other similar studies. For example, Klinge et al. [27] observed that a lightweight, multifilament polypropylene mesh exhibited less inflammation and less shrinkage compared to a standard heavyweight, monofilament polypropylene mesh when implanted in a dog model for 3–6 months. In another study, Scheidbach et al. [28] observed less shrinkage in two out of three lightweight polypropylene materials compared to a heavyweight polypropylene mesh in a porcine model after 3 months implantation.

Though others have reported differences in inflammatory foreign body response with respect to lightweight versus heavyweight polypropylenes [26], these changes were documented after several years' time in vivo. Histologic response to foreign body implants are likely an ongoing and dynamic continuum between the implanted material and the host over time. Thus, since our study was designed around short-term periods of implantation, we cannot conclude what the histologic responses would be at later timepoints. From this study, however, we can conclude that the early host response to PTFE as a monofilament knitted construct exhibits a similar histologic profile to existing lightweight and heavyweight polypropylene meshes and may prove to be a viable clinical alternative. Additionally, mkPTFE may minimize discomfort and deformative changes at the implant site. The current study represents a direct comparison between three knitted textile meshes with respect to biocompatibility in the preperitoneal space. Subsequent studies with longer time points and alternate methods of implantation will allow for further characterization of mkPTFE versus polypropylene with respect to chronic foreign body reaction and adhesiogenic properties. Our study represents an initial step in the characterization of synthetic biomaterials for ventral hernia repair in the ongoing quest to create materials that maximize durability and minimize complications.

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Fig. 1. Diagram of mesh placement and fixation in a porcine model of preperitoneal retromuscular incisional hernia repair. Mesh (8×10 cm) was centered under the 4 cm hernia defect and secured by eight transfascial sutures

Fig. 2. Biomechanical testing. The mesh-tissue specimen is secured into grips and pulled apart at a constant displacement (0.42 mm/sec). Maximum load (N), tensile strength (N/cm), and stiffness (N/mm) were recorded for each specimen

Fig. 3.

a Maximum force (N), **b** tensile strength (N/cm), **c** stiffness (N/mm) displayed for each mesh type over time (no significant differences were observed between mesh types at each individual time point, $P > 0.05$ in all cases)

Fig. 4. Macroscopic photos of mesh implants at time of harvest. Representative photos of gross specimens, viewed from the peritoneal surface. Heavyweight polypropylene (HWPP) seemed to exhibit slightly more distortion than lightweight polypropylene (LWPP) or monofilament knit polytetrafluoroethylene (mkPTFE)

Fig. 5. Inflammation, fibrosis, tissue ingrowth, and overall response scores for each mesh over time. Decreases in inflammation, increase in tissue ingrowth, and improved overall response score were observed for all mesh types, but no significant trend was observed for fibrosis (no significant differences were observed between mesh types at each individual time point, $P > 0.05$ in all cases)

Fig. 6. Sample hematoxylin and eosin (H&E) photomicrographs of HWPP, mkPTFE, and LWPP meshes at 1, 3, and 5 months, 100× magnification. Vacuolated regions represent mesh fibers

Table 1

Histologic scoring system

Score	0	1	2	3
Tissue ingrowth	None	Fair: thin bands of fibroblasts and small collagen deposits between mesh filaments	Good: moderately thick bands of fibroblasts and collagen deposits between mesh filaments	Excellent: all spaces between mesh filaments occupied by fibroblasts, collagen deposits, and capillaries

A semi-quantitative four-point scale for detecting tissue ingrowth

Table 2

Scoring system for obtaining mean overall response score, calculated from mean inflammation score and mean other response

	0	1	2	3	4
<i>Inflammation</i>					
Polymorphonuclear cells	0/hpf	1-5/hpf	6-10/hpf	Many/hpf	Abundant
Lymphocytes	0/hpf	1-5/hpf	6-10/hpf	Many/hpf	Abundant
Plasma cells	0/hpf	1-5/hpf	6-10/hpf	Many/hpf	Abundant
Macrophages	0/hpf	1-5/hpf	6-10/hpf	Many/hpf	Sheets
Giant cells	0/hpf	1-2/hpf	3-5/hpf	Many/hpf	Sheets
Necrosis	None	Limited	Minimal	Mild	Moderate
Inflammation subtotal = sum of inflammatory cell scores multiplied by 2					
Mean inflammation score (MI) = average of all inflammation subtotals					
Fibrosis	None	Minimal	Moderate	Extensive	
Fatty Infiltrate	None	Limited	Minimal	Mild	Moderate
Fibrosis + fatty infiltrate = Mean other response (MOR)					
Mean overall response (MI + MOR)					

hpf/High power field