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Biomechanical and Histologic Evaluation of Fenestrated and Nonfenestrated Biologic Mesh in a Porcine Model of Ventral Hernia Repair

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Abstract

BACKGROUND—The purpose of this study was to compare tissue incorporation and adhesion characteristics of a novel fenestrated versus nonfenestrated crosslinked porcine dermal matrix (CPDM) (Bard CollaMend) in a porcine model of ventral hernia repair.

STUDY DESIGN—Bilateral abdominal wall defects were created in 24 Yucatan minipigs, resulting in 48 defects, which were allowed to mature for 21 days. Twelve defects were repaired with fenestrated CPDM using a preperitoneal technique, 12 with fenestrated CPDM using an intraperitoneal technique, 12 with nonfenestrated CPDM using a preperitoneal technique, and 12 with nonfenestrated CPDM using an intraperitoneal technique. Half of the animals in the intraperitoneal group were euthanized after 1 month, and the other half after 3 months. Similarly, half of the animals in the preperitoneal group were euthanized after 1 month, and the other half after 6 months. Biomechanical testing and histologic evaluation were performed.

RESULTS—Intraperitoneal placement of the CPDM products resulted in significantly greater adhered area compared with preperitoneal placement ($p < 0.05$). Tissue ingrowth into preperitoneal fenestrated and nonfenestrated CPDM resulted in significantly greater incorporation strengths after 6 months compared with 1 month ($p = 0.03$ and $p < 0.0001$). Histologic analysis showed significantly greater cellular infiltration, extracellular matrix deposition, and neovascularization, with less fibrous encapsulation through the center of the fenestrations compared with all other sites evaluated, including nonfenestrated grafts.

CONCLUSIONS—Histologic findings revealed increased tissue incorporation at fenestration sites compared with nonfenestrated grafts regardless of implant location or time in vivo. However, preperitoneal placement resulted in greater incorporation strength, less adhered area, and lower adhesion scores compared with intraperitoneal placement for both fenestrated and nonfenestrated CPDM.

A variety of biologic grafts are now available from both allogenic and xenogenic sources, including dermal, pericardial, and small intestine submucosal products, some of which are

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crosslinked. Outcomes after the use of biologic graft materials depend on the tissue source, processing methods, and technique used for ventral hernia repair.¹ Physical, enzymatic, and chemical processing techniques can have substantial effects on the composition, mechanical behavior, and host response to biologic grafts.² In addition, crosslinking can also potentially affect clinical outcomes.³ The literature related to hernia repair with biologic graft materials has been limited primarily to Permacol (Tissue Science Laboratories), Surgisis (Cook Medical), and AlloDerm (Lifecell Corp),¹ and of those, only Permacol is crosslinked. So it is important to further elucidate the characteristics of crosslinked materials.

Crosslinked products are designed to resist rapid collagenase digestion and retain their tensile strength, but crosslinking may also result in partial encapsulation or minimal host cell infiltration once implanted.^{4,5} The concern with this type of response is the potential for the development of dead space at the graft-tissue interface, which can lead to an increased rate of seromas, wound complications, and/or infection.⁶⁻¹¹ A potential method to balance the negative effects associated with crosslinking with graft longevity is through fenestrating the grafts. Fenestrations would allow fluid and cells to traverse through the graft and also encourage connective tissue to be deposited at the graft surface and through the fenestrations.¹² However, there is limited knowledge as to the effect of the fenestrations on graft tensile strength, rate of graft degradation, and adhesion formation.

Our laboratory previously reported preliminary data detailing the adhesion characteristics and host response to a novel fenestrated crosslinked porcine dermal matrix (fenestrated CPDM) (CollaMend FM, CR Bard, Inc–Davol, Inc), in comparison to a nonfenestrated crosslinked porcine dermal matrix (nonfenestrated CPDM) (CollaMend, CR Bard, Inc–Davol, Inc) for the repair of abdominal wall defects.¹³ However, longer implantation times were needed to more fully characterize those products. Nonfenestrated CPDM (CollaMend) is a crosslinked porcine dermal matrix material that requires hydration with sterile normal saline or lactated Ringers solution for a minimum of 3 minutes before implantation. Details of the specific crosslinking agent that is used and the percentage of available amino acids that are crosslinked are proprietary and publicly unknown. Fenestrated CPDM (CollaMend FM) is identical to nonfenestrated CPDM except for the addition of 2-mm fenestrations spaced approximately 13 mm apart.¹³

This study compared previously reported data obtained at 1 month with data obtained at 3 and 6 months. Grafts were assessed in a porcine model of ventral incisional hernia repair, and both open intra-abdominal and open retromuscular/preperitoneal ventral hernia repair methods were represented. The tensile strengths of de novo grafts and grafts at 1 month postimplantation were compared. At 1, 3, and/or 6 months, tissue incorporation strengths of the biologic grafts were also compared through biomechanical testing. The tissue response to the biologic grafts and neovascularization was also evaluated histologically at 1, 3, and/or 6 months.

METHODS

All animals were acquired and all procedures were performed under a protocol approved by the Washington University School of Medicine Animal Studies Committee. Established protocols were followed for the humane treatment of all animals. Strict sterile conditions were maintained intraoperatively.

A model of ventral hernia repair was created using established techniques. Briefly, bilateral abdominal wall defects were created in 24 Yucatan minipigs, resulting in a total of 48 defects, which were allowed to mature for 21 days. Twelve defects were subsequently repaired with fenestrated CPDM using a preperitoneal technique, 12 were repaired with

fenestrated CPDM using an intraperitoneal technique, 12 were repaired with nonfenestrated CPDM using a preperitoneal technique, and 12 were repaired with nonfenestrated CPDM using an intraperitoneal technique. Half of the animals in the intraperitoneal group were euthanized after 1 month, and the other half after 3 months. Similarly, half of the animals in the preperitoneal group were euthanized after 1 month, and the other half after 6 months.

Ventral hernia defect creations

Bilateral, longitudinal 5-cm incisions were made through the skin, subcutaneous fat, fascia, and aponeurotic muscle layers and into the preperitoneal fat, but not through the peritoneum of the abdominal wall. The abdominal wall musculature and fascia were left open. The subcutaneous fat and areolar tissue were reapproximated with interrupted 3-0 polydioxanone (PDS) suture, and the skin was closed with interrupted subcuticular 3-0 PDS suture. Cyanoacrylate-based dermal glue was used to seal the incisions to provide a barrier to fluid and fecal contamination for at least 48 to 72 hours postoperatively. Postoperative antibiotic prophylaxis was provided as oral cefazolin dosed at 20-25 mg/kg every 12 hours for a total of 5 days.

Repair of ventral hernia defects with biologic grafts

The abdominal wall defects were grossly the same size in each animal. After 21 days, all animals developed hernias. However, the sizes of the resulting hernias were not measured. The type of repair (preperitoneal or intraperitoneal) and biologic product used (fenestrated or nonfenestrated CPDM) for repair were randomized, and 6 cm × 10 cm grafts were implanted. The manufacturer's "Instructions for Use" (<http://www.davol.com/products/soft-tissue-reconstruction/hernia-repair/biologics/collamend-fm-implants/>) were followed for handling and manipulation of the biologic grafts, including rehydration in normal saline before implantation.

Open preperitoneal or intraperitoneal ventral hernia repair

The preperitoneal repair was performed by opening the previously created abdominal wall defects and dissecting down to the underlying peritoneum, and the biologic grafts were positioned bilaterally in the preperitoneal/retromuscular space. To perform the intraperitoneal repair, a 15-cm midline laparotomy incision was made, and the grafts were centered beneath the abdominal wall defects. Grafts were oriented with the long edge (10 cm) running axially and the short edge (6 cm) running transversely, and they were secured with 8 circumferential transfascial interrupted #0 Prolene sutures placed approximately 3 cm apart and at least 1 cm from the graft edge. An overlap of 2 to 3 cm was provided circumferentially between the graft-abdominal wall interface. The hernia sac was closed with interrupted #0 PDS to eliminate excess dead space in the preperitoneal repair group. All incisions were closed primarily with a double layer of interrupted 3-0 PDS suture and sealed with cyanoacrylate-based dermal glue. Postoperative care was similar to that of the hernia creations.

Graft retrieval and adhesion assessment

Half of the animals in the intraperitoneal group were euthanized after 1 month (6 fenestrated CPDM and 6 nonfenestrated CPDM), and the other half after 3 months (6 fenestrated CPDM and 6 nonfenestrated CPDM). Similarly, half of the animals in the preperitoneal group were euthanized after 1 month (6 fenestrated CPDM and 6 nonfenestrated CPDM), and the other half after 6 months (6 fenestrated CPDM and 6 nonfenestrated CPDM).

The abdomen was opened via a midline incision, and the graft samples were visually inspected for evidence of adhesions, laxity, migration, and position of the grafts. Adhesions

of visceral structures to the repair site were recorded, and repair sites were inspected for any evidence of infection, abscess, bowel perforation, obstruction, or fistulization. The graft samples, the surrounding abdominal wall tissue, and any adhesions were then harvested en bloc. A transparent grid overlay system was used to measure the graft area (cm^2) and the area involved with adhesions. Graft surface area as a percentage of the original area at the time of implantation was calculated to determine the amount of contraction or expansion. Adhesion areas were calculated as a percentage of the total graft surface areas. The Garrard adhesion scale was used to grade the tenacity of the adhesions to the grafts.¹⁴ According to this scale, a score of 1 is assigned if no adhesions are observed; a score of 2 represents filmy adhesions that are easily disrupted manually. A score of 3 is assigned in cases of dense adhesions that require blunt dissection to remove visceral attachments from the mesh, and the most severe score of 4 is assigned when the viscera is matted to the mesh and requires sharp dissection.

Graft analysis at explantation

To allow for tensiometry evaluation and histologic processing, the implanted graft and the innermost muscle layer were separated from the remainder of the abdominal wall due to the thickness of the porcine abdominal wall. Samples were cut into a 1×4 cm strip for histologic analysis and fixed in 10% neutral buffered formalin. A 3×4 cm sample was also subjected to T-peel testing (Fig. 1). For explants at 1 month, an additional 3×4 cm piece of the graft was dissected from the abdominal wall and subjected to uniaxial tensile testing. Greater incorporation of the grafts at 3 and 6 months hindered dissection of the graft materials and prevented uniaxial tensile testing at later time points.

The graft-tissue interface underwent T-peel testing using an Instron Series 5542 Materials Testing Machine (Instron). The samples were prepared for T-peel testing by dissecting 2 opposing edges (1×3 cm) of the graft free from the underlying abdominal wall tissue, leaving a central region of tissue ingrowth (3×3 cm) intact. One free edge of the graft, measuring 1×3 cm, was secured in the upper grip of the Instron machine, and the opposing edge, containing only abdominal wall tissue and measuring 1×3 cm, was secured in the lower grip (Fig. 1). The grips were comprised of slightly textured metal plates that gripped uniformly across the entire specimen. The tissue incorporation interface (3×3 cm) was tested in tension at a rate of 0.42 mm/sec until the graft completely peeled off the underlying tissue. The maximum load sustained by the construct was recorded as incorporation strength in units of Newtons (N), where 1 Newton equals 0.225 pounds (or 1 pound equals 4.4 Newtons). All data are presented below as means $\pm$ standard error of the mean (SEM).

A second 3×4 cm sample of explanted grafts (1 month only) also underwent uniaxial tensile testing. These specimens were prepared by dissecting the grafts completely out of the abdominal wall, resulting in a specimen of the graft itself without any adherent tissue. The specimen was oriented vertically with each end secured between slightly textured metal grips. Uniaxial tensile testing was performed at a rate of 0.42 mm/sec until failure. Maximum loads sustained by the grafts were recorded as uniaxial tensile strength in units of Newtons (N).

Analysis of de novo (time-zero) grafts

De novo (time-zero) fenestrated and nonfenestrated CPDM ($n = 6$ each) were also subjected to uniaxial testing for comparison to grafts recovered after 1 month in vivo. Graft samples measuring 3×4 cm were hydrated in normal saline and then subjected to uniaxial tensile testing at a rate of 0.42 mm/sec until failure. Maximum loads sustained by the grafts were recorded as uniaxial tensile strength in units of Newtons (N).

Histologic analysis

Pieces of graft or tissue explants (1×1 cm) previously fixed in 10% neutral buffered formalin were embedded in paraffin and stained with hematoxylin and eosin. For each sample obtained from fenestrated CPDM, 2 separate slides were prepared for analysis: slide A contained a thin section directly through the center of a fenestration, and slide B included a thin section at a site distant from the fenestration. Only 1 slide was prepared for each nonfenestrated CPDM sample. A veterinary pathologist performed histologic evaluation using high-powered light microscopy (40, 100, and 200 $\times$ magnification) to evaluate the hematoxylin and eosin stained slides. A scoring system adapted from Valentin and colleagues¹⁵ and presented previously¹³ was used to evaluate the entire graft-tissue interface (5 to 10 nonoverlapping fields) at 100 $\times$. More favorable outcomes with regard to remodeling, as evidenced by cellular infiltration, host extracellular matrix (ECM) deposition, neovascularization, scaffold degradation, fibrous encapsulation, and cell types that represent low levels of inflammation were represented as higher scores within the scoring system.

Individual “site” scores were developed in order to determine if there was a histologic difference due to the presence of fenestrations in the CPDM. Each site represented a 100 $\times$ field of view. For all slides prepared from nonfenestrated CPDM and B slides prepared from fenestrated CPDM (slides distant from the fenestration), all fields of view along the implanted graft were labeled as site 0, and scores were averaged for each slide. So site 0 represented either nonfenestrated CPDM or fenestrated CPDM, evaluated far away from the fenestration (Fig. 2). For A slides prepared from fenestrated CPDM, there were multiple scoring sites: site 1 was the field of view through the center of the graft fenestration, site 2 was the field of view on either side of the fenestration (averaged together), and site 3 was the average of all other fields of view 2 or more fields away from the fenestration (Fig. 2).

Statistical analysis

For all uniaxial tensile testing analyses, an analysis of variance (ANOVA) was performed with a Fisher's LSD posttest. Statistical analysis of histologic results was performed using a Kruskal-Wallis nonparametric test with a Bonferroni correction. Ten comparisons were assumed for all data analyzed with the Bonferroni correction, and the p values reported below are adjusted by a factor of 10. A p value < 0.05 was considered statistically significant.

RESULTS

At the time of repair, examination of hernia creation sites revealed true hernia defects, measuring up to 4×2 cm, in all animals after maturation for 21 days. All hernia contents were easily reducible. At the time of euthanasia, all grafts were intact with no evidence of laxity and/or hernia recurrence.

Graft area

Rehydration is required before implantation for both fenestrated and nonfenestrated CPDM, which are both packaged in a dehydrated form. Although the manufacturer's “Instructions for Use” regarding rehydration before implantation were followed, examination at euthanasia revealed that these products continued to expand due to further rehydration in vivo after implantation. This postimplantation expansion was most notable at 1 month in the intraperitoneal location in which the fenestrated CPDM expanded to $104.7 \pm 5.5\%$ of its original area (62.8 ± 3.3 cm²) and the nonfenestrated CPDM expanded to $109.3 \pm 9.1\%$ of its original area (65.6 ± 5.4 cm²). However, at 3 months, intraperitoneal placement resulted in contraction of the grafts; mean fenestrated area was 33.5 ± 1.5 cm² and nonfenestrated

area was $37.2 \pm 9.9 \text{ cm}^2$ for intraperitoneal CPDM. The percent of original areas for both intraperitoneal CPDM products were significantly less at 3 months compared with 1 month ($p = 0.0003$) (Fig. 3). Photos showing gross findings at the time of euthanasia for 1- and 3-month intraperitoneal CPDM grafts are included (Fig. 4). Figures 4A and C show 1-month findings consistent with expansion of the grafts. Figures 4B and D show 3-month findings consistent with fibrous encapsulation of the grafts with a resultant wrinkled mesh/tissue construct. It is likely that postimplant expansion also occurred in these grafts, followed by fibrous encapsulation that resulted in the appearance of a wrinkled graft and a reduction in percent original area. When the fibrous capsules were incised, the grafts expanded laterally, indicating that the graft material was not truly reduced, but rather, was wrinkled.

At 1 and 6 months, preperitoneal placement of fenestrated and nonfenestrated CPDM resulted in contracture of the implanted products, though there was no significant difference between graft area at 1 month compared with 6 months in the preperitoneal location. At 1 month, mean fenestrated area was $41.8 \pm 1.6 \text{ cm}^2$ and nonfenestrated area was $44.2 \pm 4.9 \text{ cm}^2$ for preperitoneal CPDM. At 6 months, mean fenestrated area was $33.3 \pm 2.7 \text{ cm}^2$ and nonfenestrated area was $33.9 \pm 5.7 \text{ cm}^2$ for preperitoneal CPDM. At 1 month, preperitoneal placement resulted in a significantly decreased percent of original area compared with intraperitoneal placement for both CPDM products ($p = 0.006$), however there were no significant differences between 3-month intraperitoneal and 6-month preperitoneal percent of original graft areas ($p > 0.05$) (Fig. 3).

Adhesion area

Gross examination at 1 and 6 months revealed no adhesions to either fenestrated or nonfenestrated preperitoneal CPDM. However, intraperitoneal placement of the CPDM products resulted in a significantly greater adhered area compared with preperitoneal placement at all time points ($p < 0.05$). At 1 month, mean percent surface area covered with adhesions was $26.6 \pm 13.1\%$ for fenestrated intraperitoneal CPDM and $19.33 \pm 10.7\%$ for nonfenestrated intraperitoneal CPDM (Fig. 5). At 3 months, mean percent surface area covered with adhesions was $9.7 \pm 3.2\%$ for fenestrated intraperitoneal and $11.8 \pm 5.6\%$ for nonfenestrated intraperitoneal CPDM (Fig. 5). No significant differences were observed between 1 and 3 months for percent area covered by adhesions in the intraperitoneal location.

Adhesion score

At 1 month, intraperitoneal placement of fenestrated and nonfenestrated CPDM resulted in significantly higher mean adhesion scores (3.3 ± 0.5 and 2.8 ± 0.5 , respectively) compared with preperitoneal placement of fenestrated and nonfenestrated CPDM (1 ± 0 for both) ($p < 0.0001$ and $p = 0.0004$, respectively) (Fig. 6). At 3 months, intraperitoneal placement of fenestrated and nonfenestrated CPDM again resulted in significantly higher mean adhesion scores (2.7 ± 0.4 and 2.8 ± 0.5 , respectively) compared with preperitoneal placement of fenestrated and nonfenestrated CPDM at 6 months (1 ± 0 for both) ($p = 0.002$ and $p = 0.0008$, respectively) (Fig. 6). However, no significant differences were observed between 1 and 3 months for adhesion scores in the intraperitoneal location.

Examination of pieces of intraperitoneally placed fenestrated CPDM revealed tissue growth through the fenestrations with evidence of vascularity (Fig. 7A). Thirty-three percent (2 of 6) of the fenestrated intraperitoneal CPDM also exhibited dense adhesions to the underlying colon. Tissue growth through the fenestrations and onto the colonic surface was revealed when the edge of the colonic adhesion was sharply dissected away (Fig. 7B).

Tensile testing

As presented previously,¹³ de novo (time-zero) uniaxial tensile strength for nonfenestrated CPDM was 505.4 ± 24.6 N and for fenestrated CPDM was 176.8 ± 25.8 N. One-month uniaxial tensile strength for intraperitoneal nonfenestrated CPDM was 193.6 ± 43.9 N, intraperitoneal fenestrated CPDM was 175.6 ± 41.6 N, preperitoneal nonfenestrated CPDM was 175.0 ± 48.2 N, and preperitoneal fenestrated CPDM was 86.12 ± 20.4 N. De novo nonfenestrated CPDM demonstrated significantly greater uniaxial tensile strength compared with de novo fenestrated CPDM ($p < 0.001$), and compared with the 1-month grafts (both CPDM products in both locations) ($p < 0.001$ for all comparisons). No significant differences ($p > 0.05$) were detected between the de novo fenestrated CPDM and the 1-month uniaxial tensile strengths of the materials alone or between any of the 1-month grafts due to graft type (nonfenestrated vs fenestrated) or placement location (preperitoneal vs intraperitoneal).¹³

One-month incorporation strength (T-peel strength) for intraperitoneal nonfenestrated CPDM was 0.99 ± 0.2 N, for intraperitoneal fenestrated CPDM was 1.79 ± 0.9 N, for preperitoneal nonfenestrated CPDM was 0.07 ± 0.1 N, and for preperitoneal fenestrated CPDM was 4.96 ± 1.8 N (Fig. 8). Three-month incorporation strength for intraperitoneal nonfenestrated CPDM was 3.12 ± 1.2 N, and intraperitoneal fenestrated CPDM was 8.6 ± 5.2 N (Fig. 8). Six-month incorporation strength for preperitoneal nonfenestrated CPDM was 22.36 ± 3.3 N, and preperitoneal fenestrated CPDM was 15.68 ± 3.8 N (Fig. 8). Tissue ingrowth into both preperitoneal fenestrated and nonfenestrated CPDM resulted in significantly greater incorporation strengths after 6 months compared with 1 month ($p = 0.03$ and $p < 0.0001$, respectively).

Histology

Histologic evaluation showed that most grafts scored between 2 and 3 for cellular infiltration, indicating that cells were able to infiltrate the grafts, and some even reached the center of the graft (Tables 1 to 4). With regard to the types of cells present in the grafts, most scored between 1 and 2 and contained a mixture of both fibroblasts and inflammatory cells. ECM deposition scores varied widely, with scores ranging from 1.5 up to 3, meaning that ECM was deposited in the scaffolds, but to different extents depending on the graft type and placement location. Scaffold degradation scores were generally less than 1, which can be interpreted as only partial degradation of the grafts. Fibrous encapsulation scores varied widely from mild fibrous encapsulation up to extensive encapsulation, depending on the graft type and site. Very mild fibrous encapsulation was observed at site 1 (at the center of the fenestration) in all fenestrated grafts at all time points and in both the intraperitoneal and preperitoneal locations. Extensive encapsulation was observed at sites 0, 2, and 3 in both fenestrated and nonfenestrated grafts at all time points regardless of intraperitoneal or preperitoneal placement. Neovascularization scores also varied somewhat from scores of 1.4 up to 3, which can be interpreted as vessels present in the scaffolds near the periphery as well as up to the center of the grafts. Representative photographs are shown in Figures 9 and 10 of hematoxylin and eosin stained sections of grafts taken from both intraperitoneal and preperitoneal locations at all 3 time points. The graft material and any extracellular matrix material deposited by the host both stained eosinophilic (pink) and were differentiated by the pathologist based on morphologic features unique to each. Fibroblasts and inflammatory cells nuclei stained basophilic (blue), and again, distinctions between the cell types were made based on morphologic features unique to each type of cell.

In general, significantly greater cellular infiltration, ECM deposition, and neovascularization, along with less fibrous encapsulation, were observed at all time points through the center of the fenestrations (site 1) compared with all other sites evaluated (sites

2, 3, and 0). In most cases, significant differences were not detected between sites immediately lateral to the fenestrations (site 2) compared with sites several fields from the fenestrations (site 3), sites far away from the fenestrations (site 0), and sites on a nonfenestrated graft (site 0). However, greater cellular infiltration was observed at sites on intraperitoneal grafts immediately lateral to the fenestrations (site 2) compared with sites several fields from the fenestrations (site 3) at 1 month ($p = 0.044$). Similarly, less fibrous encapsulation was observed at sites on intraperitoneal, nonfenestrated grafts compared with immediately lateral to the fenestrations on intraperitoneal, fenestrated grafts (site 2) at 3 months ($p = 0.02$). Finally, preperitoneal grafts demonstrated greater cellular infiltration, ECM deposition, and neovascularization at the sites immediately lateral to the fenestrations (site 2) compared with sites far away from the fenestrations (site 0) at 6 months ($p < 0.05$).

Very few differences were detected due to intraperitoneal placement of the grafts compared with preperitoneal placement. Of the 6 histologic criteria evaluated, only cell types identified at the final time points showed any detectable difference due to placement location. More fibroblasts and fewer inflammatory cells were observed in the 6-month preperitoneal fenestrated grafts compared with the 3-month intraperitoneal fenestrated grafts ($p = 0.021$).

Similarly, no significant differences were detected over time. For instance, no differences were observed between 1 and 3 months for the intraperitoneal fenestrated versus intraperitoneal nonfenestrated grafts or between 1 and 6 months for the preperitoneal fenestrated versus preperitoneal nonfenestrated grafts ($p > 0.05$).

DISCUSSION

Some studies have shown that crosslinked products are more resistant to degradation in the host tissue environment than noncrosslinked products, potentially providing a more durable repair.^{16,17} So, hernia recurrence is one of the biggest concerns surrounding the use of noncrosslinked products.¹⁸ However, the crosslinking process that allows these products to remain intact for a longer period of time^{16,17} can also lead to decreased cellular infiltration, increased dead space, and increased wound complications.⁶⁻¹¹

One possible way for a crosslinked biologic graft to exhibit the versatility of a biologic graft and yet avoid these wound complications is to manufacture it with fenestrations.³ Fenestrations would allow fluid, cells, and new blood vessels to traverse through the graft and also encourage connective tissue to be deposited through the fenestrations.¹² Thus far, there is limited information in the literature as to the effect of the fenestrations on graft tensile strength, rate of graft degradation, and adhesion formation. This study was undertaken in an effort to better characterize biomechanical properties, host response, and adhesion characteristics of crosslinked materials with and without fenestrations. Although biologic grafts are commonly used in infected or contaminated ventral hernia repairs, the model used in this study was limited to clean cases and as such, did not seek to evaluate the performance of these grafts in the presence of bacteria.

Our results showed significantly lower de novo uniaxial strength for fenestrated CPDM compared with nonfenestrated CPDM. However, these differences can likely be attributed to the position of fenestrations at the edge of the CPDM during testing. Fenestrations near the edge of the CPDM created stress concentrations and allowed the grafts to fail at a lower load compared with nonfenestrated CPDM.

Tensile testing of biologic graft materials recovered after 1 month in vivo revealed very similar tensile strengths among all grafts regardless of the presence of fenestrations, indicating that the fenestrations did not significantly weaken the integrity of the grafts in

vivo. Placement location also did not appear to have an impact on the integrity of the graft materials in the early postoperative period because tensile strengths were similar for both preperitoneal and intraperitoneal fenestrated and nonfenestrated CPDM evaluated after 1 month in vivo. However, it should be noted that the tensile strength of all specimens harvested after 1 month in vivo (both fenestrated and nonfenestrated CPDM in both intraperitoneal and preperitoneal locations) were significantly reduced compared to the de novo tensile strength of nonfenestrated CPDM ($p < 0.001$ for all comparisons). This reduction in graft strength is likely the result of enzymatic digestion of the ECM proteins comprising the graft.

Though differences in tissue incorporation strength (ie, T-peel strength) were not detected at the earlier time points, preperitoneal grafts exhibited significantly greater tissue incorporation strengths after 6 months versus 1 month for both fenestrated and nonfenestrated CPDM, indicating that tissue ingrowth develops into stronger incorporation over longer periods of time. In addition, tissue incorporation strengths for nonfenestrated grafts were also stronger at 6 months in the preperitoneal location compared with 3 months in the intraperitoneal location, indicating that placement location may improve tissue incorporation even for grafts without fenestrations. Fenestrations may also provide additional benefits in the preperitoneal space in which the grafts are in contact with vascularized tissue without the threat of adhesions as in the intraperitoneal space.

Histologic analysis also revealed significantly increased ECM deposition and neovascularization with less fibrous encapsulation within the fenestrations (site 1) compared with all other sites, regardless of implantation site or time point. These histologic scores suggest increased incorporation of the fenestrated CPDM compared with the nonfenestrated CPDM. In addition, sites immediately lateral to the fenestrations (site 2) exhibited very similar characteristics compared with sites farther away from the fenestrations and sites found on nonfenestrated grafts (sites 0 and 3). These results suggest that the presence of fenestrations did not accelerate degradation of the grafts outward from the fenestration and did not significantly affect the overall longevity of the grafts in vivo. Our results also revealed another potential benefit related to the fenestrations. Evidence of vascularized tissue growing through the fenestrations was observed for both implant locations (Fig. 7). Vascular tissue growth extending through the fenestrations may help to decrease seroma formation by allowing for absorption of seromatous fluid.

It should also be noted that in the intraperitoneal location, the fenestrations did allow for tissue growth through the fenestrations and onto the side of the graft in contact with abdominal viscera. In 2 cases this resulted in dense colonic adhesions (Garrard adhesion grade of 4), with an area of adhesion of 100% for each aforementioned graft. However, this tissue growth did not lead to significantly increased adhered area or adhesion scores for intraperitoneal fenestrated CPDM samples compared with nonfenestrated CPDM at either 1 or 3 months. Significantly fewer adhesions (ie, zero) were observed for preperitoneal placement of both fenestrated and nonfenestrated CPDM. So it is advisable to place CPDM in the preperitoneal location whenever possible. This practice would limit the potential complications associated with adhesions and subsequent adhesiolysis during subsequent operations.

CONCLUSIONS

Histologic findings revealed increased tissue incorporation and vascular integration at fenestration sites compared with nonfenestrated grafts regardless of implant location or time in vivo. However, preperitoneal placement resulted in greater incorporation strength, less adhered area, and lower adhesion scores compared with intraperitoneal placement for both

fenestrated and nonfenestrated CPDM. Overall, the findings of this study indicate that fenestrations allow increased tissue incorporation and vascular integration without accelerating the degradation of the graft and leading to weakening and loss of graft integrity. However, it should be noted that fibrous encapsulation and wrinkling of the grafts were also observed.

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Abbreviations and Acronyms

CPDM	crosslinked porcine dermal matrix
ECM	extracellular matrix
PDS	polydioxanone

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Figure 1.

T-peel schematic where the free edge of mesh was secured in the upper grip and the free edge of abdominal wall tissue was secured in the lower grip. The fixation interface (3×3 cm) was then tested in tension.

Figure 2.

(A) Nonfenestrated CPDM histologic scoring site 0. (B) Fenestrated CPDM histologic scoring sites 0 (away from fenestration), 1 (at fenestration), 2 (field of view on either side of the fenestration), and 3 (all other fields of view, 2 or more fields away from the fenestration). CPDM, crosslinked porcine dermal matrix.

Figure 3.

Percent of original area of grafts at explant: comparisons of fenestrated and nonfenestrated CPDM in intraperitoneal (1 and 3 months) and preperitoneal (1 and 6 months) locations. Six grafts represented per bar. Data reported as mean $\pm$ SEM. The percent of original areas for both intraperitoneal CPDM products were significantly less at 3 months compared with 1 month ($p = 0.0003$). At 1 and 6 months, preperitoneal placement of fenestrated and nonfenestrated CPDM resulted in contracture of the implanted products, though there was no significant difference between graft areas at 1 month compared with 6 months in the preperitoneal location ($p > 0.05$). CPDM, crosslinked porcine dermal matrix.

Figure 4. Gross examination. (A) Intraperitoneal fenestrated at 1 month; (B) intraperitoneal fenestrated at 3 months with contraction and wrinkling; (C) intraperitoneal nonfenestrated at 1 month; and (D) intraperitoneal nonfenestrated at 3 months with contraction and wrinkling.

Figure 5.

Adhesed area: comparisons of fenestrated and nonfenestrated CPDM in intraperitoneal (1 and 3 months) and preperitoneal (1 and 6 months) locations. Six grafts are represented per bar. Data are reported as mean $\pm$ SEM. Intraperitoneal placement of the CPDM products resulted in a significantly greater adhesed area compared with preperitoneal placement at all time points ($p < 0.05$). In addition, no significant differences were observed between 1 and 3 months ($p > 0.05$) in the intraperitoneal location. CPDM, crosslinked porcine dermal matrix.

Figure 6.

Adhesion score: comparisons of fenestrated and nonfenestrated CPDM in intraperitoneal (1 and 3 months) and preperitoneal (1 and 6 months) locations. Six grafts are represented per bar. Data are reported as mean $\pm$ SEM. At 1 month, intraperitoneal placement of fenestrated and nonfenestrated CPDM resulted in a significantly higher mean adhesion score compared with preperitoneal placement of fenestrated and nonfenestrated CPDM ($p < 0.0001$ and $p = 0.0004$, respectively). At 3 months, intraperitoneal placement of fenestrated and nonfenestrated CPDM again resulted in a significantly higher mean adhesion score compared with preperitoneal placement of fenestrated and nonfenestrated CPDM at 6 months ($p = 0.002$ and $p = 0.0008$, respectively). No significant differences were observed between 1 and 3 months for the adhesion scores in the intraperitoneal location. CPDM, crosslinked porcine dermal matrix.

Figure 7. Gross examination. (A) Close-up of intraperitoneal fenestrated graft with radial growth pattern through fenestrations. (B) Tissue growth through fenestration to colonic surface.

Figure 8.

Incorporation strength (T-peel force): comparisons of fenestrated and nonfenestrated CPDM in intraperitoneal (1 and 3 months) and preperitoneal (1 and 6 months) locations. Six grafts are represented per bar. Data are reported as mean $\pm$ SEM. Tissue ingrowth into both preperitoneal fenestrated and nonfenestrated CPDM resulted in significantly greater incorporation strengths after 6 months compared with 1 month ($p = 0.03$ and $p < 0.0001$, respectively). Tissue ingrowth into nonfenestrated CPDM also resulted in significantly greater incorporation strengths after 6 months in the preperitoneal location compared with 3 months in the intraperitoneal location ($p < 0.0001$).

Figure 9.

Histologic examinations: (A) 1-month intraperitoneal fenestrated (40 $\times$), (B) 1-month intraperitoneal fenestrated (100 $\times$), (C) 3-month intraperitoneal fenestrated (40 $\times$), (D) 3-month intraperitoneal fenestrated (100 $\times$), (E) 1-month intraperitoneal nonfenestrated (40 $\times$), (F) 1-month intraperitoneal nonfenestrated (100 $\times$), (G) 3-month intraperitoneal nonfenestrated (40 $\times$), (H) 3-month intraperitoneal nonfenestrated (100 $\times$).

Figure 10.

Histologic examinations: (A) 1-month preperitoneal fenestrated (40 $\times$), (B) 1-month preperitoneal fenestrated (100 $\times$), (C) 6-month preperitoneal fenestrated (40 $\times$), (D) 6-month preperitoneal fenestrated (100 $\times$), (E) 1-month preperitoneal nonfenestrated (40 $\times$), (F) 1-month preperitoneal nonfenestrated (100 $\times$), (G) 6-month preperitoneal nonfenestrated (40 $\times$), (H) 6-month preperitoneal nonfenestrated (100 $\times$).

Table 1Histology Results for Grafts Implanted for 1 Month in the Preperitoneal Location (Mean $\pm$ SEM)

Histology	Fenestrated, site 1	Fenestrated, site 2	Fenestrated, site 3	Fenestrated, site 0	Nonfenestrated, site 0
Cellular infiltration	2.86 $\pm$ 0.14	2.44 $\pm$ 0.14	2.14 $\pm$ 0.10	2.18 $\pm$ 0.17	2.22 $\pm$ 0.19
Cell types	1.86 $\pm$ 0.14	1.83 $\pm$ 0.15	1.80 $\pm$ 0.12	1.37 $\pm$ 0.16	1.59 $\pm$ 0.03
ECM deposition	3.00 $\pm$ 0.00	2.26 $\pm$ 0.17	1.88 $\pm$ 0.13	1.89 $\pm$ 0.21	2.07 $\pm$ 0.20
Scaffold degradation	2.50 $\pm$ 0.34	0.37 $\pm$ 0.09	0.21 $\pm$ 0.04	0.42 $\pm$ 0.11	0.46 $\pm$ 0.14
Fibrous encapsulation	2.46 $\pm$ 0.37	0.14 $\pm$ 0.14	0.07 $\pm$ 0.07	0.50 $\pm$ 0.34	0.46 $\pm$ 0.31
Neovascularization	3.00 $\pm$ 0.00	2.17 $\pm$ 0.17	1.75 $\pm$ 0.13	1.82 $\pm$ 0.20	1.89 $\pm$ 0.26

ECM, extracellular matrix.

Table 2Histology Results for Grafts Implanted for 1 Month in the Intraperitoneal Location (Mean $\pm$ SEM)

Histology	Fenestrated, site 1	Fenestrated, site 2	Fenestrated, site 3	Fenestrated, site 0	Nonfenestrated, Site 0
Cellular infiltration	2.67 $\pm$ 0.22	2.55 $\pm$ 0.14	1.87 $\pm$ 0.08	1.97 $\pm$ 0.22	2.20 $\pm$ 0.19
Cell types	1.58 $\pm$ 0.06	1.30 $\pm$ 0.11	1.19 $\pm$ 0.06	1.41 $\pm$ 0.14	1.54 $\pm$ 0.05
ECM deposition	2.67 $\pm$ 0.22	2.09 $\pm$ 0.25	1.53 $\pm$ 0.11	1.73 $\pm$ 0.27	2.03 $\pm$ 0.21
Scaffold degradation	2.63 $\pm$ 0.22	0.55 $\pm$ 0.10	0.26 $\pm$ 0.05	0.38 $\pm$ 0.11	0.36 $\pm$ 0.10
Fibrous encapsulation	1.91 $\pm$ 0.46	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.51 $\pm$ 0.31	0.00 $\pm$ 0.00
Neovascularization	2.67 $\pm$ 0.22	1.96 $\pm$ 0.23	1.38 $\pm$ 0.10	1.54 $\pm$ 0.25	1.85 $\pm$ 0.20

ECM, extracellular matrix.

Table 3Histology Results for Grafts Implanted for 3 Months in the Intraperitoneal Location (Mean $\pm$ SEM)

Histology	Fenestrated, Site 1	Fenestrated, Site 2	Fenestrated, Site 3	Fenestrated, Site 0	Nonfenestrated, Site 0
Cellular infiltration	3.00 $\pm$ 0.00	2.42 $\pm$ 0.15	2.31 $\pm$ 0.08	2.16 $\pm$ 0.18	2.53 $\pm$ 0.18
Cell types	1.63 $\pm$ 0.07	1.56 $\pm$ 0.03	1.61 $\pm$ 0.02	1.68 $\pm$ 0.10	1.61 $\pm$ 0.05
ECM deposition	3.00 $\pm$ 0.00	2.25 $\pm$ 0.18	2.20 $\pm$ 0.09	1.96 $\pm$ 0.20	2.39 $\pm$ 0.24
Scaffold degradation	3.00 $\pm$ 0.00	0.63 $\pm$ 0.14	0.46 $\pm$ 0.05	0.63 $\pm$ 0.23	0.62 $\pm$ 0.13
Fibrous encapsulation	2.75 $\pm$ 0.25	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.30 $\pm$ 0.20	0.17 $\pm$ 0.11
Neovascularization	3.00 $\pm$ 0.00	2.13 $\pm$ 0.18	2.05 $\pm$ 0.09	1.89 $\pm$ 0.20	2.15 $\pm$ 0.25

ECM, extracellular matrix.

Table 4Histology Results for Grafts Implanted for 6 Months in the Preperitoneal Location (Mean $\pm$ SEM)

Histology	Fenestrated, Site 1	Fenestrated, Site 2	Fenestrated, Site 3	Fenestrated, Site 0	Nonfenestrated, Site 0
Cellular infiltration	3.00 $\pm$ 0.00	2.65 $\pm$ 0.14	2.77 $\pm$ 0.04	2.42 $\pm$ 0.17	2.57 $\pm$ 0.10
Cell types	2.17 $\pm$ 0.14	1.77 $\pm$ 0.05	1.84 $\pm$ 0.03	1.71 $\pm$ 0.06	1.74 $\pm$ 0.07
ECM deposition	3.00 $\pm$ 0.00	2.65 $\pm$ 0.14	2.71 $\pm$ 0.04	2.35 $\pm$ 0.19	2.48 $\pm$ 0.08
Scaffold degradation	3.00 $\pm$ 0.00	0.63 $\pm$ 0.13	0.60 $\pm$ 0.05	0.67 $\pm$ 0.11	0.63 $\pm$ 0.06
Fibrous encapsulation	3.00 $\pm$ 0.00	0.13 $\pm$ 0.13	0.07 $\pm$ 0.04	0.01 $\pm$ 0.01	0.45 $\pm$ 0.26
Neovascularization	3.00 $\pm$ 0.00	2.60 $\pm$ 0.14	2.55 $\pm$ 0.06	2.19 $\pm$ 0.17	2.31 $\pm$ 0.07

ECM, extracellular matrix.