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Evaluation of fenestrated and non-fenestrated biologic grafts in a porcine model of mature ventral incisional hernia repair

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Abstract

Introduction—The purpose of this study is to compare the tissue incorporation of a novel fenestrated and non-fenestrated crosslinked porcine dermal matrix (CPDM) (CollaMend™, Davol Inc., Warwick, RI) in a porcine model of ventral hernia repair.

Methods—Bilateral abdominal wall defects were created in 12 Yucatan minipigs and repaired with a preperitoneal or intraperitoneal technique 21 days after hernia creation. Animals were randomized to fenestrated or non-fenestrated CPDM for $n = 6$ pieces of each graft in the preperitoneal or intraperitoneal location. All animals were sacrificed at 1 month. Adhesion characteristics and graft contraction/growth were measured by the Garrard adhesion grading scale and transparent grid overlay. Histological analysis of hematoxylin and eosin (H&E)-stained slides was performed to assess graft incorporation. Tissue incorporation strength was measured by a T-peel tensile test. The strength of explanted CPDM alone and de novo CPDM was measured by a uniaxial tensile test using a tensiometer (Instron, Norwood, MA) at a displacement rate of 0.42 mm/s. Statistical significance ($P < 0.05$) was determined for histological analysis using a Kruskal–Wallis non-parametric test with a Bonferroni correction, and for all other analyses using a two-way analysis of variance (ANOVA) with a Bonferroni post-test or a Kruskal–Wallis non-parametric test with a Dunn’s post-test.

Results—Intraperitoneal placement of fenestrated CPDM resulted in a significantly higher area of adhesions and adhesion score compared to the preperitoneal placement of fenestrated CPDM ($P < 0.05$). For both preperitoneal and intraperitoneal placement, histological findings demonstrated greater incorporation of the graft due to the fenestrations. No significant differences were detected in the uniaxial tensile strengths of the graft materials alone, either due to the graft type (non-fenestrated vs. fenestrated) or due to the placement location (preperitoneal vs. intraperitoneal). The incorporation strength (T-peel force) was significantly greater for fenestrated compared to non-fenestrated CPDM when placed in the preperitoneal location ($P < 0.01$). The incorporation strength was also significantly greater for fenestrated CPDM placed in the preperitoneal location compared to fenestrated CPDM placed in the intraperitoneal location ($P < 0.05$).

Conclusions—Fenestrations in CPDM result in greater tissue incorporation strength and lower adhesion area and score when placed in the preperitoneal location. Fenestrations in CPDM allow for greater tissue incorporation without accelerating graft degradation. Fenestrations may be placed in CPDM while still allowing adequate graft strength for intraperitoneal and preperitoneal hernia repairs at 1 month in a porcine model.

Keywords

Crosslinked; Biologic mesh; Fenestrated; Hernia repair; Dermal matrix; Acellular dermal matrix

Introduction

Surgeons face a challenging situation when attempting to close abdominal wall defects that are complicated by contamination, multiple reoperative abdomen, inadequate abdominal wall for closure, or loss of domain. Operative strategies must be employed to minimize perioperative complications [1, 2]. The use of permanent synthetic products in heavily contaminated wounds is associated with very high rates of infection, leading to recurrence or necessitating mesh removal in up to 50–90% of cases [3, 4]. Thus, biologic grafts have been developed in an effort to provide strength, flexibility, host incorporation, and infection resistance in such ventral hernia repairs at high risk for wound complications [5].

Biologic products are available from allogenic and xenogenic sources (bovine and porcine) and vary with respect to the site of harvest (dermis, pericardium, or small intestine submucosa [SIS]), sterilization process, and possible crosslinking. Of all possible product differences, chemical crosslinking appears to have the largest effect on host response [6]. Products that are not crosslinked are resorbed by the host and replaced by endogenous host connective tissue [7-9]. Non-crosslinked biologic products serve as a scaffold for cellular ingrowth, and neocollagen is deposited throughout the entire graft [6]. Crosslinked biologic materials have been shown to behave as functionally non-porous, resulting in a host response limited to the graft surface, similar to totally microporous (pore size $<10\text{ }\mu\text{m}$) synthetic mesh [7, 8]. These crosslinked products are partially encapsulated, minimally infiltrated by host cells [7,8], and, therefore, remain structurally intact longer than non-crosslinked materials [10, 11]. Crosslinking, thus, allows the potential for a more durable repair, as it is not rapidly broken down in vivo. However, like the microporous synthetic meshes, crosslinked biologic products can develop dead space at the graft–tissue interface, resulting in increased rates of seromas, wound complications, and/or infection [12-17].

Crosslinked materials with fenestrations behave as functionally porous prosthetics, allowing fluid and cells to traverse through the fenestrations and for connective tissue to be deposited at both the graft surface and through the fenestrations [6]. Fenestrations, thus, change the host's response to the crosslinked material to a mixed encapsulation and incorporation response similar to that generated by a macroporous synthetic mesh [6]. However, the effect of the fenestrations on graft tensile strength, rate of graft degradation, and adhesion formation has not been defined.

The purpose of this study was to evaluate a novel fenestrated crosslinked porcine dermal matrix (fenestrated CPDM) (CollaMend™ FM), as compared to a non-fenestrated crosslinked porcine dermal matrix (non-fenestrated CPDM) (CollaMend™) for the repair of abdominal wall defects. In this study, grafts were assessed with respect to tissue attachment and healing response in a porcine model of mature ventral incisional hernia repair. Open intra-abdominal and open retromuscular/preperitoneal ventral hernia repair methods are represented. Graft tensile strengths are compared for de novo graft and at 1 month post-implantation. The incorporation strength of the biologic grafts to the abdominal wall tissue was also compared at 1 month following graft implantation. Furthermore, microscopic tissue incorporation and markers of tissue response to the biologic graft were evaluated histologically at 1 month.

Methods

This study was performed under a protocol (# 20070298) approved by the Washington University School of Medicine Animal Studies Committee. All animals were housed, fed, and handled according to established protocols for humane animal treatment currently in practice at our institution. All animals were operated on under strict sterile conditions. Twelve female Yucatan minipigs 3–4 months of age were acquired for use in this study.

Bilateral abdominal wall defects were created in 12 Yucatan minipigs and repaired with a preperitoneal or intraperitoneal technique 21 days after hernia creation, representing a model of mature ventral hernia repair. Animals were randomized to receive fenestrated or non-fenestrated CPDM for $n = 6$ pieces of each graft in the preperitoneal or intraperitoneal location. Fenestrated CPDM was manufactured with fenestrations measuring ~2 mm in diameter and spaced ~13 mm apart.

Creation of mature ventral hernia defects

Two 5-cm longitudinal incisions were made through the skin, subcutaneous fat, fascia, and aponeurotic muscle layers and into the preperitoneal fat, but not through the peritoneum to the right and left of the midline abdominal wall. While leaving the abdominal wall musculature and fascia open, the subcutaneous fat and areolar tissue were reapproximated with interrupted 3-0 PDS suture, and the skin was closed with interrupted subcuticular 3-0 PDS suture. The incisions were then sealed with a cyanoacrylate-based dermal glue to provide a barrier to fluid and fecal contamination for at least 48–72 h postoperatively. Each pig received postoperative antibiotic prophylaxis with oral cefazolin at 20–25 mg/kg Q12 h for a total of 5 days.

Graft repair of mature ventral hernia defects

The abdominal wall defects were left untreated for 21 days to achieve mature status and then repaired with biologic grafts. Animals were randomized preoperatively as to the type of repair (preperitoneal or intraperitoneal) and biologic product utilized (fenestrated or non-fenestrated CPDM) for repair. All grafts measured 6×10 cm at implantation. The biologic grafts were rehydrated in normal saline, and handled and manipulated per the manufacturer's "Instructions for Use".

Open preperitoneal or intraperitoneal ventral hernia repair

To perform the preperitoneal repair, the previously created wounds were opened and dissected down to the underlying peritoneum and the biologic grafts were positioned bilaterally in the preperitoneal/retromuscular space. The intraperitoneal repair was performed through a 15-cm midline laparotomy incision and the grafts were centered beneath the abdominal wall defects. In both types of repair, the grafts were oriented with the long edge (10 cm) running axially and the short edge (6 cm) running transversely. The graft was secured with eight circumferential transfascial interrupted sutures of #0 Prolene placed approximately 3–4 cm apart and at least 1 cm from the graft edge. The repair allowed for 2–3 cm of overlap (graft–abdominal wall interface) circumferentially. In the preperitoneal repair group, the hernia sac was closed with interrupted #0 PDS to eliminate excess dead space. For both repair groups, subcutaneous fat and areolar tissue were reapproximated with interrupted 3-0 PDS suture, and the skin was closed with interrupted subcuticular 3-0 PDS suture. The incisions were sealed with a cyanoacrylate-based dermal glue. Postoperative care was similar to that of the hernia creations.

Graft retrieval and adhesion assessment

After 1 month, all animals were sacrificed. Following sacrifice, the abdomen was opened via a midline incision and the graft samples visually inspected for evidence of adhesions. The repairs were then carefully inspected for laxity, migration, and position. Any visceral structures adhered to the repair site were recorded, along with any evidence of infection, abscess, bowel perforation, obstruction, or fistulization. The graft samples and surrounding abdominal wall tissue with any adhesions were then harvested en bloc. The graft area as well as the area involved with adhesions were measured in cm^2 using a transparent grid overlay system. The adhesion area was calculated as a percentage of the total surface area. Adhesions to the graft were graded according to the Garrard adhesion scale [18] (Table 1). The graft surface area as a percentage of the original dimensions at the time of implantation (60 cm^2) was calculated to determine the amount of contraction or expansion.

Explanted graft analysis

Due to the thickness of the porcine abdominal wall, the implanted graft and innermost muscle layer were separated from the remainder of the abdominal wall to allow tensiometry evaluation and further histologic processing. Samples were cut into three separate pieces: a $1 \times 4\text{-cm}$ strip for histological analysis, a $3 \times 4\text{-cm}$ piece for T-peel testing (Fig. 1), and a $3 \times 4\text{-cm}$ piece of the explanted graft free of surrounding tissue for uniaxial testing. Histology samples were fixed in 10% neutral buffered formalin.

Immediately following explant, the graft–tissue interface underwent T-peel testing using an Instron Series 5542 Materials Testing Machine (Instron, Norwood, MA). Screw-action grips were utilized to grip the samples during testing. The free edge of the graft, measuring $1 \times 3 \text{ cm}$, was secured in the upper grip, and the free edge of the abdominal wall tissue, measuring $1 \times 3 \text{ cm}$, was secured in the lower grip (Fig. 1). The fixation interface ($3 \times 3 \text{ cm}$) was tested in tension at a rate of 0.42 mm/s until the graft completely peeled off the incorporated tissue. The maximum load sustained by the construct was recorded as the incorporation strength in units of Newtons (N), where 1 Newton equals 0.225 lb (or 1 lb equals 4.4 Newtons). The data are presented below as means $\pm$ standard error of the mean (SEM).

The $3 \times 4\text{-cm}$ pieces of explanted graft underwent uniaxial testing using the tensiometer (Instron) at a rate of 0.42 mm/s until failure. Maximum loads sustained by the grafts were recorded as uniaxial tensile strength in units of Newtons (N). Data are presented below as means $\pm$ standard error of the mean (SEM).

De novo (time-zero) graft analysis

De novo (time-zero) fenestrated and non-fenestrated CPDM ($n = 6$ each) were also subjected to uniaxial testing. Grafts were cut into $3 \times 4\text{-cm}$ pieces and hydrated in normal saline as per manufacturer specifications. The samples were then subjected to uniaxial tensile testing (Instron) at a rate of 0.42 mm/s until failure. Maximum loads sustained by the grafts were recorded as uniaxial tensile strength in units of Newtons (N). Data are presented below as means $\pm$ standard error of the mean (SEM).

To further characterize the biologically derived materials, six de novo specimens ($n = 6$) of both fenestrated and non-fenestrated CPDM were cut into $8 \times 8\text{-cm}$ squares and subjected to ball-burst testing (Instron). A standard test method (ASTM #D3787-07) was utilized to test the strength of the fenestrated and non-fenestrated CPDM by applying a 2.54-cm-diameter (1-inch) stainless steel ball at a constant rate of 300 mm/min (12 inches/min) until the materials burst. The tensile strength (N/cm) and percent elongation (%) of the materials were subsequently calculated based on the maximum load and extension measured at the bursting point.

Histologic analysis

Samples fixed in 10% neutral buffered formalin were cut into 1 × 1-cm pieces, embedded in paraffin, and stained with hematoxylin and eosin. For each sample obtained from fenestrated CPDM, two separate slides were prepared for analysis: slide “A” contained a thin section directly through a fenestration and slide “B” included a thin section at a site distant from the fenestration. For each sample obtained from non-fenestrated CPDM, only one slide was prepared. Histological evaluation was performed by a board-certified veterinary pathologist using high-powered light microscopy (40, 100, and 200× magnification) to evaluate the H&E-stained slides. The entire graft–tissue interface (5–10 non-overlapping fields) was evaluated at 200× using a scoring system adapted from Valentin et al. [19] (Table 2). Higher scores within the scoring system represent 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.

In order to determine if there was a histologic difference due to the presence of fenestrations in the CPDM, individual “site” scores were developed. Each site represented a 2009 field of view. For all slides prepared from non-fenestrated CPDM and “B” slides prepared from fenestrated CPDM, all fields of view along the implanted graft were labeled as site “0” and scores were averaged for each slide. Site “0” was, thus, either non-fenestrated CPDM or fenestrated CPDM, evaluated away from fenestration (Fig. 2). For “A” slides prepared from fenestrated CPDM, there were multiple scoring sites: site “1” was the field of view with tissue ingrowth through the fenestration, site “2” was the field of view on either side of the fenestration, averaged together, and site “3” was all other fields of view, two or more fields away from the fenestration, averaged together (Fig. 2). Ten hypotheses were utilized to compare histologic results with regard to the multiple graft types and histologic site combinations (Tables 3 and 4).

Statistical analysis

For all analyses, excluding ball-burst testing and histology, statistical analysis was performed using either a two-way analysis of variance (ANOVA) with a Bonferroni post-test or a Kruskal–Wallis non-parametric test with a Dunn’s post-test. Analysis of ball-burst results was performed via an unpaired, two-tailed *t*-test. Statistical analysis of histological results was performed using a Kruskal–Wallis non-parametric test with a Bonferroni correction. A value of $P < 0.05$ was considered to be statistically significant.

Results

Before the repair of hernia defects, all animals were examined. All hernia creation sites had resultant defects measuring up to 4 × 2 cm. These were true defects with easily reducible contents. At sacrifice, all animals were examined for graft laxity and/or hernia recurrence. All grafts were grossly intact with no evidence of laxity at the repair sites. No hernia recurrences were noted at 1 month for either fenestrated or non-fenestrated CPDM.

Adhesions and graft area

Gross examination at sacrifice revealed no adhesions to either fenestrated or non-fenestrated preperitoneally placed CPDM. The percentage surface area with adhesions for intraperitoneal grafts was $26.2 \pm 13.1\%$ for fenestrated CPDM and $19.33 \pm 10.7\%$ for non-fenestrated CPDM (Fig. 3). Intraperitoneal placement of the CPDM products resulted in a significantly higher adhered area compared to preperitoneal placement ($P < 0.05$). Intraperitoneal placement of fenestrated and non-fenestrated CPDM resulted in a significantly higher adhesion score (3.3 ± 0.5 and 2.8 ± 0.5 , respectively) compared to

preperitoneal placement of fenestrated and non-fenestrated CPDM (1 ± 0 for both) ($P < 0.001$ and 0.01 , respectively) (Fig. 4). Of note, gross examination of pieces of intraperitoneally placed fenestrated CPDM demonstrated tissue growth through the fenestrations onto the surface of the graft (Fig. 5). Two of the six samples of fenestrated intraperitoneal CPDM had dense adhesions to the underlying colon. When the edge of the colonic adhesion was sharply dissected away, tissue growth through the fenestrations onto the colonic surface was evidenced, with a resultant thickened colonic wall (Fig. 6).

Intraperitoneal placement of fenestrated and non-fenestrated CPDM resulted in expansion of the graft. The mean graft area was $62.8 \pm 3.3 \text{ cm}^2$ for fenestrated and $65.6 \pm 5.4 \text{ cm}^2$ for non-fenestrated intraperitoneal CPDM, and the percentages of the original graft area were $104.7 \pm 5.5\%$ for fenestrated and $109.3 \pm 9.1\%$ for non-fenestrated intraperitoneal CPDM. Preperitoneal placement of fenestrated and non-fenestrated CPDM resulted in contracture of the implanted products. The areas were $41.8 \pm 1.6 \text{ cm}^2$ for fenestrated and $44.2 \pm 4.9 \text{ cm}^2$ for non-fenestrated intraperitoneal CPDM, and the percentage-areas of the original graft area were $69.6 \pm 2.7\%$ for fenestrated and $73.6 \pm 8.2\%$ for non-fenestrated preperitoneal CPDM, respectively. Preperitoneal placement location resulted in a significantly decreased graft area at 1 month explant compared to intraperitoneal placement ($P < 0.01$) (Fig. 7).

Tensile testing

De novo (time-zero) uniaxial tensile strengths were $505.4 \pm 24.6 \text{ N}$ for non-fenestrated CPDM and $176.8 \pm 25.8 \text{ N}$ for fenestrated CPDM. One month uniaxial tensile strengths were $193.6 \pm 43.9 \text{ N}$ for intraperitoneal non-fenestrated CPDM, $175.6 \pm 41.6 \text{ N}$ for intraperitoneal fenestrated CPDM, $175.0 \pm 48.2 \text{ N}$ for preperitoneal non-fenestrated CPDM, and $86.12 \pm 20.4 \text{ N}$ for preperitoneal fenestrated CPDM (Fig. 8). De novo non-fenestrated CPDM demonstrated significantly greater uniaxial tensile strength compared to de novo fenestrated CPDM ($P < 0.001$). De novo non-fenestrated CPDM also demonstrated greater uniaxial tensile strength compared to the 1 month uniaxial tensile strength of all implanted grafts (fenestrated and non-fenestrated CPDM in both preperitoneal and intraperitoneal locations) ($P < 0.001$ for all comparisons). No significant differences ($P > 0.05$) were detected in the 1 month uniaxial tensile strengths of the materials alone, either due to graft type (non-fenestrated vs. fenestrated) or due to placement location (preperitoneal vs. intraperitoneal) (Fig. 8).

De novo ball-burst tensile strengths were $110.3 \pm 7.8 \text{ N/cm}$ for non-fenestrated CPDM and $119.3 \pm 26.5 \text{ N/cm}$ for fenestrated CPDM (Fig. 9). The percent elongation for de novo non-fenestrated and fenestrated CPDM were 17.96 ± 0.6 and $56.22 \pm 8.1\%$, respectively (Fig. 9). No significant differences were detected between the ball-burst tensile strengths of fenestrated and non-fenestrated CPDM ($P > 0.05$). Fenestrated CPDM exhibited significantly greater percent elongation compared to non-fenestrated CPDM ($P = 0.008$) (Fig. 9).

The incorporation strength (T-peel force) was $0.99 \pm 0.2 \text{ N}$ for intraperitoneal non-fenestrated CPDM, $1.79 \pm 0.9 \text{ N}$ for intraperitoneal fenestrated CPDM, $0.07 \pm 0.1 \text{ N}$ for preperitoneal non-fenestrated CPDM, and $4.96 \pm 1.8 \text{ N}$ for preperitoneal fenestrated CPDM (Fig. 10). The incorporation strength (T-peel force) was significantly greater for fenestrated CPDM compared to non-fenestrated CPDM when placed in the preperitoneal location ($P < 0.01$) (Fig. 10). The incorporation strength was also significantly greater for fenestrated CPDM placed in the preperitoneal location compared to fenestrated CPDM placed in the intraperitoneal location ($P < 0.05$) (Fig. 10).

Histology

For the preperitoneal placement group, the majority of scoring categories confirmed hypotheses #1, 2, 4, 5, 8, 9, and 10 to be true, where $P < 0.05$ proved non-equivalence and $P > 0.05$ suggested NO difference (Table 3). For the intraperitoneal placement group, the majority of scoring categories confirmed hypotheses #1, 2, 4, 5, 8, 9, and 10 to be true, where $P < 0.05$ proved non-equivalence and $P > 0.05$ suggested NO difference (Table 4). However, these findings were not as consistent as those shown in the preperitoneal placement location.

For both preperitoneal and intraperitoneal placement groups, histologic findings demonstrated that there was greater incorporation of the graft due to the fenestrations. Fenestrations allowed tissue growth directly through the material (site 1) (Fig. 11). This effectively increased incorporation without causing accelerated degradation of the graft. Histologic scores for sites directly bordering the fenestration (site 2) and sites away from the fenestrations (sites 0 and 3) were equivalent to non-fenestrated CPDM (site 0). These findings were true in both preperitoneal and intraperitoneal repair locations.

Discussion

Permanent synthetic mesh materials are contraindicated in the setting of contaminated abdominal wall defects [20-22]. In this setting, absorbable synthetic mesh has been used to re-establish short-term abdominal wall integrity. However, in these patients, multiple procedures are generally required for abdominal wall closure, patients are at increased risk for enteric fistula formation, and the vast majority develop a recurrent ventral hernia [23, 24]. Biologic materials have, thus, been developed to aide in abdominal wall closure where synthetic products are contraindicated or prone to further complications. The biologic graft often acts as a “biologic dressing” for those patients who develop a subsequent ventral hernia in long-term follow up, and require further reinforcement.

The vast majority of published analyses of biologic materials for hernia repair are limited to the use of Allo-Derm (Lifecell Corp., Branchburg, NJ), Surgisis (Cook Medical, Bloomington, IN), and Permacol (Tissue Science Laboratories, Hampshire, UK) [25]. Despite the growing number of publications concerning these materials, a recent review of the literature revealed that level 1 evidence to support the use of any biologic materials for ventral hernia repair does not exist [25]. Nevertheless, surgeons continue to use biologic grafts for ventral hernia repair at an accelerating rate. In many cases, this is due to the fact that there is “no other good alternative”. Many surgeons are using biologic products for the closure of complex abdominal wounds in the presence of contamination and/or other appropriate conditions [26]. However, some surgeons are using biologic grafts in clean wounds where a synthetic mesh would be a cheaper, more durable alternative, but, more importantly, supported by clinical outcomes studies [27, 28].

Non-fenestrated CPDM (CollaMend™, Davol Inc., Warwick, RI) is a commercially available lyophilized acellular biologic graft with both collagen and elastin fiber components. It is chemically crosslinked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) and requires hydration with sterile normal saline or lactated ringers solution for a minimum of 3 min prior to implantation. Fenestrated CPDM (CollaMend™ FM, Davol Inc., Warwick, RI) is a novel fenestrated biologic graft processed identically to non-fenestrated CPDM until the final manufacturing step, where it is die-cut to create fenestrations measuring ~2 mm in diameter and spaced ~13 mm apart.

There is a paucity of literature regarding the use of non-fenestrated CPDM (CollaMend™). However, the limited available publications to date raise concern over the number of wound

complications related to seroma formation [17]. Similarly, in an in vivo primate model, non-fenestrated CPDM showed considerable contraction, pleating, and graft hardening, consistent with the histologic response which showed an inflammatory response limited to the graft perimeter without matrix penetration [29]. Their study suggests that chemical crosslinking is responsible for the preclusion of immune cell penetration and overall non-incorporation of non-fenestrated CPDM [29]. This is consistent with previous descriptions of crosslinked biologic materials behaving in a similar manner to totally microporous (pore size $<10\ \mu\text{m}$) synthetic mesh [7, 8]. Since crosslinked products are encapsulated and minimally infiltrated by host cells [7, 8], they are less subject to chemical breakdown in the host tissue environment and may, therefore, provide a more durable repair [10, 11].

Manufacturing crosslinked materials with fenestrations has been previously proposed to create a durable biologic prosthetic that would exhibit the versatility of a biologic product and, yet, avoid the associated wound complications discussed earlier [6]. The results of our study demonstrate increased incorporation with fenestrated CPDM. Incorporation strength as evidenced by the T-peel test is significantly greater for fenestrated CPDM compared to non-fenestrated CPDM when placed in the preperitoneal location. Although the incorporation strength for preperitoneal fenestrated CPDM is relatively low ($4.96 \pm 1.8\ \text{N}$), it represents a notable improvement compared to preperitoneal non-fenestrated CPDM, which was found to have very low incorporation strength ($0.07 \pm 0.1\ \text{N}$). Histologic analysis also demonstrates that there is greater incorporation of the graft due to the fenestrations. Tissue ingrowth directly through the graft at the fenestration sites is clearly evident on gross inspection (Fig. 11). These findings will likely correlate with a clinically significant reduction in wound complications with regard to decreased seroma formation with fenestrated CPDM use in vivo.

The ability to achieve greater incorporation strength by manufacturing CPDM with fenestrations does not significantly reduce the structural integrity of the graft product, nor does it accelerate graft degradation. Our results demonstrate that these important qualities were not sacrificed. Although there is a statistically significant decrease in de novo (time-zero) graft uniaxial strength ($176.8 \pm 25.8\ \text{N}$ for de novo fenestrated CPDM), these differences can likely be attributed to the position of the fenestrations on the test article during testing. If fenestrations happened to fall near the edge of the test article, a stress concentration in that region was likely to cause the specimen to fail at a lower load. This is confirmed by equivalent ball-burst tensile strengths of the de novo fenestrated and non-fenestrated CPDM, 119.30 ± 26.5 and $110.30 \pm 7.8\ \text{N/cm}$, respectively. It is also important to note that at 1 month, fenestrated and non-fenestrated CPDM demonstrated equivalent graft strength, between 86.12 ± 20.4 and $193.6 \pm 43.9\ \text{N}$. At intra-abdominal pressures of 20 kPa (150 mm Hg), the abdominal wall must exhibit a tensile strength on the order of 16–32 N/cm when reinforced or replaced with a mesh material, respectively [30]. At least initially, both of these materials possess an appropriate strength for hernia repair applications with ball-burst tensile strengths approximately seven times greater than the forces generated by the pressure inside the human abdomen. The fenestrations also result in a material that is significantly more extensible than the non-fenestrated CPDM with percent elongations at burst of 56.22 ± 8.1 and $17.96 \pm 0.6\%$, respectively. This increased extensibility could translate into reduced foreign body sensation, alleviating some patient discomfort, but too much extensibility could result in “bulging” or recurrence over the long term. Regarding graft degradation, histologic analysis reveals no accelerated degradation of the graft, as histologic scores for sites directly bordering the fenestration (site 2) and sites away from the fenestrations (sites 0 and 3) are equivalent to non-fenestrated CPDM (site 0). These findings are consistent in both preperitoneal and intraperitoneal repair locations. While these findings demonstrate resistance to degradation after 1 month in vivo, the structural integrity of fenestrated CPDM after a longer implantation time also needs to be evaluated.

Although there is clear evidence of vascularized tissue growth through the fenestrations, this does not correlate to significantly increased adhered area or adhesion scores for intraperitoneal fenestrated CPDM samples compared to non-fenestrated CPDM. However, intraperitoneal placement resulted in significantly more adhesions than the preperitoneal placement of either CPDM product. In order to limit the potential of complications relating to adhesions and the direct exposure of visceral organs to prosthetic materials, CPDM should be placed in the preperitoneal location when possible.

Fenestrated and non-fenestrated CPDM are packaged in a dehydrated form and require rehydration prior to implantation. Despite following the manufacturer's "Instructions for Use" regarding rehydration prior to implantation, we found that these products continue to expand, which is likely due to further rehydration after implantation. This was most notable in the intraperitoneal location, where the percentage of the original graft area was $104.7 \pm 5.5\%$ for fenestrated and $109.3 \pm 9.1\%$ for non-fenestrated CPDM. Preperitoneal placement of fenestrated and non-fenestrated CPDM resulted in contracture of the implanted products due to encapsulation. However, the products were rugated, and when the fibrous capsules were incised, they expanded laterally. Although the total graft area outside of the fibrous encapsulation was not able to be measured for preperitoneal products, it is likely that these products also expanded due to further rehydration after implantation.

Conclusions

Our study demonstrates that fenestrations in crosslinked porcine dermal matrix (CPDM) result in greater tissue incorporation strength when placed in the preperitoneal location. Fenestrations in CPDM allow for greater tissue incorporation without accelerating graft degradation. Fenestrations placed in CPDM allow for all of the afore-mentioned benefits while still allowing adequate graft integrity for intraperitoneal and preperitoneal hernia repairs at 1 month in a porcine model.

Intraperitoneal placement of fenestrated and non-fenestrated CPDM results in less graft contracture and equivalent graft strength; however, it also results in lower incorporation strength, higher adhered area, and higher adhesion scores compared to preperitoneal placement of fenestrated and non-fenestrated CPDM. Complications of potential adhesions should be considered before intraperitoneal placement of either CPDM product. Further evaluation needs to be performed to evaluate graft integrity and remodeling after long-term implantation. Evaluation in a model of contamination could also be undertaken to simulate circumstances in which biologic grafts should be utilized. Clinical evaluation also needs to be performed to evaluate for expected translational results regarding decreased seroma formation with fenestrated CPDM.

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Fig. 1.

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

Fig. 2.

a Non-fenestrated crosslinked porcine dermal matrix (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, two or more fields away from the fenestration)

Fig. 3.
Adhesed area comparisons of fenestrated and non-fenestrated CPDM in preperitoneal and intraperitoneal locations

Fig. 4. Adhesion score comparisons of fenestrated and non-fenestrated CPDM in preperitoneal and intraperitoneal locations

Fig. 5.
a Gross examination of intraperitoneal fenestrated graft with radial growth pattern through fenestrations. **b** Close-up of growth through fenestration

Fig. 6.
Tissue growth through fenestration to colonic surface

Fig. 7.
Area of graft at explants, comparisons of fenestrated and non-fenestrated CPDM in preperitoneal and intraperitoneal locations

Fig. 8. Uniaxial tensile strengths of de novo (time-zero), intraperitoneal, and preperitoneal graft

Fig. 9. De novo (time-zero) fenestrated and non-fenestrated CPDM ball-burst testing comparisons: **a** tensile strength and **b** percent elongation

Fig. 10. Incorporation strength (T-peel force): preperitoneal and intraperitoneal comparisons and fenestrated and non-fenestrated CPDM comparisons

Fig. 11.
Histological examination at 40× magnification of tissue growth through graft fenestration

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

Table 2

Histologic scoring system

Score	0	1	2	3
Cellular infiltration	Zero cells in contact with scaffold	Cells contact periphery, no penetration into scaffold	Cells infiltrate scaffold, but none reach center	Cells penetrate into center of scaffold
Cell types ("inflammatory cells" include neutrophils, macrophages, and foreign body giant cells)	Inflammatory cells present, no fibroblasts	Primarily inflammatory cells, few fibroblasts	Primarily fibroblasts, few inflammatory cells	Fibroblasts only, no inflammatory cells
Host extracellular matrix (ECM) deposition	No host ECM deposition	Host ECM deposited at periphery of scaffold	Host ECM deposited inside scaffold, but not at the center	Host ECM deposited inside scaffold, including the center
Scaffold degradation	Original scaffold intact, borders clearly demarcated	Scaffold partially degraded, layers separated by cells, blood vessels, host tissue, etc.	Scaffold extremely degraded, difficult to distinguish scaffold from host tissue	Scaffold completely degraded, no evidence of original scaffold
Fibrous encapsulation	Extensive encapsulation (50–100%) of periphery)	Moderate encapsulation (25–50% of periphery)	Mild encapsulation (<25% of periphery)	No fibrous encapsulation
Neovascularization	Zero blood vessels present	Vessels present at scaffold periphery, no penetration into scaffold	Vessels infiltrate scaffold but none reach center of scaffold	Vessels penetrate into center of scaffold

Table 3

Results of preperitoneal histological analysis, listed by hypotheses

Hypotheses	Cellular infiltration	Cell types	ECM deposition	Scaffold degradation	Fibrous encapsulation	Neo-vascularization
1 site 1 is <i>different</i> from site 2			Yes *	Yes *	Yes *	Yes *
2 site 1 is <i>different</i> from site 3	Yes *		Yes *	Yes *	Yes *	Yes *
3 site 2 is <i>different</i> from site 3						
4 site 1 is <i>different</i> from site 0 on the same piece of fenestrated CPDM	Yes *		Yes *	Yes *	Yes *	Yes *
5 site 1 is <i>different</i> from site 0 on a piece of non-fenestrated CPDM			Yes *	Yes *	Yes *	Yes *
6 site 2 is <i>different</i> from site 0 on the same piece of fenestrated CPDM						
7 site 2 is <i>different</i> from site 0 on a piece of non-fenestrated CPDM						
8 site 3 is <i>NOT different</i> from site 0 on the same piece of fenestrated CPDM	Yes **	Yes **	Yes **	Yes **	Yes **	Yes **
9 site 3 is <i>NOT different</i> from site 0 on a piece of non-fenestrated CPDM	Yes **	Yes **	Yes **		Yes **	Yes **
10 site 0 on a piece of fenestrated CPDM is <i>NOT different</i> from site 0 on a piece of non-fenestrated CPDM	Yes **	Yes **	Yes **	Yes **	Yes **	Yes **

*
 $P < 0.05$ **
 $P > 0.05$

Table 4

Results of intraperitoneal histological analysis, listed by hypotheses

Hypotheses	Cellular infiltration	Cell types	ECM deposition	Scaffold degradation	Fibrous encapsulation	Neo-vascularization
1 site 1 is <i>different</i> from site 2				Yes *	Yes *	
2 site 1 is <i>different</i> from site 3	Yes *		Yes *	Yes *	Yes *	Yes *
3 site 2 is <i>different</i> from site 3	Yes *					
4 site 1 is <i>different</i> from site 0 on the same piece of fenestrated CPDM				Yes *		Yes *
5 site 1 is <i>different</i> from site 0 on a piece of non-fenestrated CPDM			Yes *	Yes *	Yes *	Yes *
6 site 2 is <i>different</i> from site 0 on the same piece of fenestrated CPDM						
7 site 2 is <i>different</i> from site 0 on a piece of non-fenestrated CPDM						
8 site 3 is <i>NOT different</i> from site 0 on the same piece of fenestrated CPDM	Yes **	Yes **	Yes **	Yes **		Yes **
9 site 3 is <i>NOT different</i> from site 0 on a piece of non-fenestrated CPDM	Yes **		Yes **	Yes **	Yes **	Yes **
10 site 0 on a piece of fenestrated CPDM is <i>NOT different</i> from site 0 on a piece of non-fenestrated CPDM	Yes **	Yes **	Yes **	Yes **	Yes **	Yes **

*
 $P < 0.05$ **
 $P > 0.05$