MULTILAYERED SMALL INTESTINAL SUBMUCOSA IS INFERIOR TO AUTOLOGOUS BOWEL FOR LAPAROSCOPIC BLADDER AUGMENTATION

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ABSTRACT

Purpose: Bladder augmentation is most commonly performed with ileum. However, porcine small intestinal submucosa has been reported as a substitute for bowel for incorporation into the urinary tract. We assessed the feasibility and long-term 12-month results of laparoscopic bladder augmentation with ileum or multilayered small intestinal submucosa (Cook Biotech, Spencer, Indiana) in a porcine model.

Materials and Methods: We performed laparoscopically assisted hemicystectomy and bladder augmentation in 24 female Yucatan mini-pigs using an ileal segment (12) or multilayered small intestinal submucosa (12). The followup protocol included anesthetic bladder capacity, renal ultrasonography and serum chemistry. At 3, 6 and 12 months, respectively, 4 animals per group were scheduled for sacrifice and pathological analysis.

Results: Despite longer anastomotic time in the multilayered small intestinal submucosa group (120 versus 91 minutes, p = 0.026) total operative time was similar in the 2 groups. In each group bladder capacity increased with time but by 12 months bladder capacity was significantly better in the bowel than in the small intestinal submucosa group (825 versus 431 cc, p = 0.016). At 3 months pathological evaluation revealed that the multilayered regenerated bladder patch had shrunk and by 6 months it was replaced by dense calcified scar tissue. Long-term 6 and 12-month bladder capacity in the small intestinal submucosa group was the result of the regeneration of native bladder with exclusion of the whole multilayered patch in the majority of cases.

Conclusions: Laparoscopic bladder augmentation using multilayered small intestinal submucosa produced functional and pathological results inferior to those of bowel at 12-month followup in a porcine model.

KEY WORDS: bladder; urinary diversion; ileum; intestine, small; swine

Bladder augmentation is performed to increase capacity and reduce intravesical pressure in small, contracted and/or hyperactive bladders. Although almost all segments of the gastrointestinal tract can be used for bladder augmentation, the ileum is the least contractile segment and, therefore, it may be the segment of choice. Ileocystoplasty has good results with voiding and continence achieved in up to 77% of patients with intractable detrusor instability and in 82% with tonic instability or poor compliance. However, augmentation cystoplasty is major surgery associated with significant complications and investigators have sought alternatives to bowel incorporation into the urinary tract in an attempt to reduce morbidity. Native tissues, such as autografts or ureterocystoplasty, as well as various free organic grafts for bladder augmentation have been studied. Materials such as skin, fascia, preserved bladder, peritoneum, omentum, lyophilized human dura, pericardium, placenta, reversed seromuscular grafts and acellular tissue matrix have been applied. However, most of these new alternatives are still limited in applicability or have yet to show long-term efficacy. The recent use of porcine small intestinal submucosa seems promising. Nevertheless, enterocystoplasty remains the gold standard for bladder augmentation.

Laparoscopic surgery can offer several advantages over open surgery, including decreased hospital stay, rapid return to preoperative daily activity and better cosmetic results. We have previously reported our results showing the feasibility and acceptable early 3-month results of laparoscopic ileocystoplasty. We now present up to 12 months of followup in a series of laparoscopic bladder augmentation using porcine multilayered small intestinal submucosa or autologous bowel (ileum).

MATERIALS AND METHODS

Surgical procedure. The experimental protocol was approved by the Methodist Hospital of Indiana animal care and use committee. The animals were allowed a minimum 2-day acclimatization period before surgery. Oral antibiotics were administered preoperatively and continued for 10 days postoperatively, while intraoperatively 1 gm. cefazolin was given intravenously. All animals were rendered unconscious with an intramuscular injection of ketamine (15 to 20 mg./kg.) and xylazine (2 mg./kg.). Each animal was then intubated and anesthetized with 1% to 3% isoflurane for the remainder of surgery. Oral antibiotics were administered preoperatively and continued for 10 days postoperatively, while intraoperatively 1 gm. cefazolin was given intravenously. All animals were rendered unconscious with an intramuscular injection of ketamine (15 to 20 mg./kg.) and xylazine (2 mg./kg.). Each animal was then intubated and anesthetized with 1% to 3% isoflurane for the remainder of surgery. Our technique of laparoscopically assisted transverse hemicystectomy with ileocystoplasty and laparoscopic
transverse hemicyrstectomy with bladder augmentation using multilayered small intestinal submucosa has been previously reported.\textsuperscript{12,13} Bladder augmentation using autologous bowel. In 12 Yucatan mini-pigs with a mean weight of 66 pounds (range 53 to 76) bladder augmentation was performed with a 4 laparoscopic port technique. We placed 3, 12 mm. ports at the umbilicus, right and left lower abdominal quadrants with an additional 5 mm. port placed at a right suprapubic site. After pneumoperitoneum was achieved a 12 cm. segment of terminal ileum that reached the bladder without tension was grasped with endoscopic bowel clamps and positioned below the umbilical trocar site. The grasped bowel was then externalized through a 3 cm. umbilical incision. The ileal segment for bladder augmentation was isolated using gastrointestinal anastomosis staplers, bowel continuity was reestablished and the isolated ileal segment was opened and irrigated with a U-shaped patch fashioned. The bladder patch was repositional back into the abdominal cavity and pneumoperitoneum was reestablished. Subsequently a 4 × 4 cm. segment of bladder dome was excised and the size of the resected bladder segment was measured extracorporeally. The bowel patch was anastomosed to the bladder with a running 4-zero polyglactin the Endo-Stitch device (fig. 1, a). The bladder was distended with saline to assess urinary leaks. Points of leakage were closed with additional figure-of-8 sutures using the Endo-Stitch device (fig. 1, a). The bladder was drained with a 14Fr Foley catheter for 7 days.

Multilayered small intestinal submucosa bladder augmentation. In 12 Yucatan mini-pigs weighing a mean of 72 pounds (range 54 to 85) a 6 × 8 cm. multilayered small intestinal submucosa patch was used instead of bowel for bladder augmentation. The anastomosis to the bladder was performed with 5-zero polyglactin the Endo-Stitch device (fig. 1, b). The bladder was drained with a 14Fr Foley catheter for 7 days.

Preoperative evaluation and followup. Evaluations were performed initially and every 3 months depending on survival duration. Each evaluation included complete blood count, serum chemistry and renal ultrasound. All animals had normal preoperative complete blood count, serum chemistry and renal ultrasound. All animals survived the initial surgical procedure and there were no intraoperative complications. Average operative time was similar in the bowel and small intestinal submucosa groups (227 minutes, range 188 to 290 and 211, range 157 to 300, respectively, p = 0.41). However, average anastomotic time in the bowel augmentation group was significantly shorter than in the small intestinal submucosa group (91 minutes, range 60 to 140 versus 120, range 64 to 170, p = 0.026). No significant difference in somatic growth, serum electrolytes or serum creatinine was seen in the groups. Bladder capacity declined initially in each group but at a longer followup of 12 months the bowel group had significantly larger bladder capacity than the small intestinal submucosa augment group (p = 0.016, table 1).

Results of bladder augmentation with ileum. Several complications developed in the postoperative period (table 2). An animal in the 6-month survival arm was sacrificed at 6 weeks due to anastomotic leakage and 1 was treated for acute cystitis with gross hematuria. In 2 animals in the 3-month survival arm right hydronephrosis at harvest was due to a mid and a distal ureteral stricture, respectively. All other animals had normal kidneys on ultrasound and at harvest. Mild to moderate intra-abdominal and pelvic adhesion formation was noted in all cases but in only 1 with 12 months of survival was partial small bowel obstruction suspected. No animals showed clinical evidence of bowel obstruction.

Pathological review revealed a normal transition between ileal mucosa and urothelium, and the bowel mucosa was unremarkable (fig. 2, a). In addition, no bladder stones or visible sutures were seen. However, in 4 of 12 animals minimal osseous metaplasia at the suture line was detected with Mayer’s hematoxylin and eosin. The patch was also assessed for inflammation, scarring, re-epithelialization, revascularization, and muscle and nerve tissue ingrowth into the patch.

Statistics. Baseline measurements in the 2 treatment groups were analyzed by the t test to determine whether the groups were equivalent. Measurements at 3, 6 and 12 months in the 2 treatment groups were compared by ANOVA with the baseline value as the covariate and the treatment group as the grouping factor. In no case was the covariate significant and, therefore, we present ANOVA results. A significant interaction term on ANOVA indicates that the change with time differed in the 2 treatment groups. Significance was considered at p < 0.05.

**Table 1. Bladder capacity using anesthesia at sacrifice in the bowel and multilayered porcine small intestinal submucosa groups**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Av. Ml. at 40 Cm.</th>
<th>No. Subjects</th>
<th>Av. Ml. at 40 Cm.</th>
<th>No. Subjects</th>
<th>Av. Ml. at 40 Cm.</th>
<th>No. Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water ± SD</td>
<td>Water ± SD</td>
<td>Water ± SD</td>
<td>Water ± SD</td>
<td>Water ± SD</td>
<td>Water ± SD</td>
<td>Water ± SD</td>
</tr>
<tr>
<td>Bowel</td>
<td>550 ± 114</td>
<td>12</td>
<td>348 ± 134</td>
<td>3</td>
<td>350 ± 37</td>
<td>4</td>
</tr>
<tr>
<td>Small intestinal submucosa</td>
<td>563 ± 157</td>
<td>12</td>
<td>220 ± 101</td>
<td>3</td>
<td>455 ± 250</td>
<td>4</td>
</tr>
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</table>

Note markedly improved bladder capacity in the bowel group by 12 months of followup.
the small intestinal submucosa group underwent sacrifice at 5 weeks due to anastomotic leakage (table 2). In addition, a greater degree of intra-abdominal adhesions was noted in this group compared with the bowel group. At 3-month harvest the patch was still distinct but it had shrunken with an average size reduction of 74% (48 to 12.49 cm²). In all 4, 12-month and in 2 of the 4, 6-month sacrificed animals a dense ossified plaque (osseous metaplasia) was found at the site of patch placement. Histological evaluation of the small intestinal submucosa regenerated bladder in the 3-month survivor animals, in which the multilayered small intestinal submucosa was still discernible, revealed minimal ingrowth of smooth muscle and vessels, minimal inflammation and a thin layer of urothelium coating the patch (fig. 2, b).

**DISCUSSION**

The feasibility of laparoscopic bladder augmentation with autologous bowel has previously been demonstrated in animal studies and human case series. In our porcine animal model we noted that laparoscopic ileocystoplasty resulted in significant improvement in bladder capacity with time and no deterioration in renal function (table 1). In contrast, long-term followup revealed that functional and pathological results of multilayered small intestinal submucosa bladder augmentation were inferior to those of autologous bowel bladder augmentation. In fact, in the majority of cases of 6 and 12-month multilayered small intestinal submucosa bladder augmentation recorded bladder capacity was the result of native bladder regeneration since small intestinal submucosa was excluded from the bladder lumen. In addition, a greater degree of intra-abdominal adhesions was noted with small intestinal submucosa bladder augmentation, which we hypothesize may have been secondary to subclinical urinary extravasation or an intense inflammatory reaction directed at the small intestinal submucosa patch.

Our results with multilayered small intestinal submucosa are supported by the recent study of Sofer et al, who reported bone formation in 1 of 5 pigs and calcifications in 3 of 5 that underwent segmental ureteral replacement with multilayered small intestinal submucosa. Despite regeneration of the urothelium and muscular cells over the graft in these cases the multilayered small intestinal submucosa replaced neoureters were embedded in dense fibrotic inflammatory tissue with the ureteral lumina occluded. However, severe inflammation and scarring are not universal after small intestinal submucosa application since other researchers have not observed this finding with small intestinal submucosa incorporation into the pig urinary tract. Portis et al reported only minimal adhesion formation with single layer small intestinal submucosa bladder patches at an early sacrifice point of 12 weeks postoperatively. Liatsikos et al reported regeneration of all ureteral layers without scar tissue or calcification at 7 weeks of followup in 8 pigs that underwent laparoscopic ureteral replacement with multilayered small intestinal submucosa.

Extensive experience with small intestinal submucosa incorporation into the urinary tract has been reported that highlights this material as acceptable biomaterial for use in the urinary tract. Kropp and Vaught et al used single layer small intestinal submucosa successfully for bladder augmentation immediately after partial cystectomy in rats. At 48 weeks all 3 layers of the normal bladder were present, including functional cholinergic and purinergic innervation similar to normal rat bladder, while graft shrinkage was not observed. A later report of these authors of small intestinal submucosa for bladder augmentation in dogs indicated no significant shrinkage of the patch at followup as long as 15 months. In contrast, we found that porcine bladder augmentation with multilayered small intestinal submucosa resulted in a reduction in patch size with time with the patch later engulfed and replaced by abundant scar formation. In addition to graft shrinkage, another concern in our study was extensive osseous metaplasia in 6 of the 12 long-term survival animals, that is those with greater than 6 months of survival. Osseous metaplasia has been previously reported with small intestinal submucosa, peritoneum, myocutaneous flaps, free fascial grafts, pericardial grafts and lyophilized human dura grafts. The finding of osseous metaplasia with small intestinal submucosa incorporation into the urinary tract is not limited to studies in the pig model. Pope et al reported osseous metaplasia and dystrophic calcification in 14 of 22 dogs (64%) that underwent bladder augmentation with small intestinal submucosa. Osseous metaplasia in

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**Table 2. Complications of laparoscopic bladder augmentation**

<table>
<thead>
<tr>
<th>Complication</th>
<th>No. Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowel:</td>
<td>12</td>
</tr>
<tr>
<td>Anastomotic leakage</td>
<td>1</td>
</tr>
<tr>
<td>Ureteral stricture</td>
<td>2</td>
</tr>
<tr>
<td>Partial small bowel obstruction</td>
<td>1</td>
</tr>
<tr>
<td>Cystitis, hematuria</td>
<td>1</td>
</tr>
<tr>
<td>Minimal osseous metaplasia</td>
<td>4</td>
</tr>
<tr>
<td>Total No.</td>
<td>9</td>
</tr>
<tr>
<td>Small intestinal submucosa:</td>
<td></td>
</tr>
<tr>
<td>Anastomotic leakage</td>
<td>1</td>
</tr>
<tr>
<td>Severe osseous metaplasia</td>
<td>6</td>
</tr>
<tr>
<td>Total No.</td>
<td>7</td>
</tr>
</tbody>
</table>

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More complications were seen in the bowel group but the degree of osseous metaplasia in the small intestinal submucosa group was significantly more pronounced than in the bowel group.

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Fig. 2. Histological study. a, at bowel-bladder junction note clear demarcation of bowel and bladder sections with absent inflammation. b, in multilayered small intestinal submucosa regenerated bladder note thin urothelium overlying patch with paucity of smooth muscle and vascular ingrowth. Reduced from ×40.
this dog study comprised less than 5% of the graft area in 7 animals and 10% to 40% in another 7. All osseous metaplasia was noted in the submucosa but a few cases had extension into the mid zone and in 1 there was almost transmural osseous metaplasia. The majority of grafts with osseous metaplasia in that dog study were noted more than 6 weeks postoperatively, suggesting that animal studies with too short a followup do not show this finding. However, osseous metaplastic replacement of small intestinal submucosa appears to be primarily limited to studies in which small intestinal submucosa is incorporated into the urinary tract and is in constant contact with urine. Currently we can only hypothesize that an inflammatory reaction to the slowly degrading thick multilayered small intestinal submucosa patch in the presence of urine directed tissue remodeling toward severe fibrosis and later bone formation.

There are multiple differences in the harvesting and processing of single and multilayered small intestinal submucosa but none that can readily explain the difference in results in previous studies and our study. The original small intestinal submucosa used by Kropp et al was harvested by hand and not thermally sterilized. In contrast, the much thicker multilayered small intestinal submucosa formed by 4 layers of small intestinal submucosa oriented at 90 degrees to each subsequent layer that was used in our study was mechanically harvested and later sterilized, freeze-dried and vacuum sealed. No agents were used during manufacture to laminate the small intestinal submucosa layers because the layers adhere to each other only by partial degradation via proteolysis (Cook Urological, personal communication). However, a notable difference in our and previous studies that may partially explain our adverse results with multilayered small intestinal submucosa is the longer duration of bladder drainage (7 versus 2 days) in our series. It is possible that partial collapse of the multilayered small intestinal submucosa with bladder decompression led to an intense inflammatory reaction around the large foreign body with fixation of the collapsed patch within an inflammatory mass, reapproximation of the cut edges of the native bladder and exclusion of the patch from the bladder lumen with time (fig. 4). However, pathological review of bladders harvested at 3 months revealed that the multilayered small intestinal submucosa comprised a portion of the bladder wall, which suggests that exclusion of the patch occurred beyond the early postoperative period.

CONCLUSIONS

Our long-term animal study with up to 12 months of followup showed significantly better functional and pathological results when laparoscopic bladder augmentation was performed with autologous bowel than with multilayered small intestinal submucosa. The presence of extensive adhesion formation, exclusion of the multilayered small intestinal submucosa patch from the bladder and severe osseous metaplasia with multilayered small intestinal submucosa incorporation into the urinary tract is concerning. We do not recommend the use of multilayered small intestinal submucosa for bladder augmentation.

Tracy Robinson and Stephanie Derdak provided assistance, Dr. Naomi Fineberg, Department of Medicine, Indiana University School of Medicine performed the statistical analysis and Dr. Martin Kaefer, Department of Urology, Indiana University School of Medicine assisted in manuscript preparation.

REFERENCES


