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Use of Reconstructed Small Intestine Submucosa for Urinary Tract Replacement

Xie, Hua; Shaffer, Brian S.*; Wadia, Yasmin; Gregory, Kenton W.

Author Information

From the Oregon Medical Center, *Department of Urology, Providence St. Vincent Medical Center, Portland, Oregon.
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Reprint requests: Hua Xie, MD, PhD, Oregon Medical Laser Center, Providence St. Vincent Medical Center, 9205 SW Barnes Rd, Portland, OR 97225.

Abstract

We used reconstructed SIS (ReSIS), a photocrosslinked biomaterial, to create grafts in various shapes and sizes. Sheets of ReSIS were placed in 14 swine to repair bladder defects, and ReSIS tubes were placed in six swine to replace a segment of excised ureter. Histologic analysis of the bladder repair revealed transitional urothelial cells lining the ReSIS by 1 week. After 2 weeks, fibroblasts and mononuclear cells had infiltrated the ReSIS, neovascularization had occurred, and the urothelial lining was more complex, containing multiple cell layers. After 4 weeks, a definite submucosa was present and the ReSIS was starting to degrade. An initial muscular regeneration was demonstrated at 12 weeks. No foreign body reaction, calcification, or sedimentation was noted in any animal. The ureteral implants showed identical histologic changes, without obstruction or leakage of the replaced segment. The ReSIS allowed rapid urothelial regeneration, ingrowth of new blood vessels, and the orderly deposition and organization of new collagen. Our study demonstrates that the photocrosslinking technique used to create larger sheets and tubes of this biomaterial (ReSIS) does not detract from the positive attributes of the SIS and should improve its usefulness in accomplishing larger bladder augmentations and ureter replacements.

Varying defects of the urinary tract due to cancer, trauma, infection, iatrogenic injuries, or congenital abnormalities may lead to a variety of problems, from quality of life issues to renal failure. Until now, the gastrointestinal tract has been the chief source of material for bladder and ureter substitution, despite complications such as metabolic abnormalities, infections, perforation, urolithiasis, mucus production, and malignancy. There is no adequate urinary tract substitute material. 1

For decades, clinicians and scientists have been attempting to reconstruct the urinary system with different materials. Synthetic biomaterials such as polyvinyl and gelatin sponges, Teflon, Silastic, and Gore-Tex, as well as naturally derived materials such as lyophilized dura, peritoneum, pericardium, placenta, fascia, and collagen matrix have been unsuccessful for urinary tract replacement. 2,3 The most common reasons for failure are foreign body reaction, stone formation, infection, fistula, graft shrinkage, metabolic bone formation, and the lack of normal tissue incorporation into the graft. 2 In addition to these problems, no biomaterial has been able to mimic structural function such as contraction of the bladder or peristalsis within the ureter. The ideal graft material should be replaced by normal host tissue. Such a biodegradable urinary tract substitute would provide a scaffold for regrowth of normal urinary tract tissue while providing a structurally intact low pressure vessel or reservoir until such regrowth had occurred. Recently, grafts of small intestine submucosa (SIS) have been shown to have regenerative capabilities in various sites. 4 SIS is a biodegradable, collagen based, nonimmunogenic material harvested from the submucosal layer of porcine intestine. Animal experiments have demonstrated that SIS can be used with great success as a xenograft for urinary bladder wall regeneration. 5 However, its narrow size and thin composition may limit its urologic applications. 6 Recently, we developed a multilayered, reconstructed SIS (ReSIS) using a photocrosslinking method. ReSIS can be fashioned into tubes for ureteral replacement, or into large sheets for significant bladder augmentation. This study investigated the feasibility of using ReSIS as a material for urinary tract replacement.

Materials and Methods

Preparation of the ReSIS

Harvesting of small intestine submucosa (SIS) has been previously described. 5 The SIS segments were decellularized in a solution of 2 mM SDS and 0.1 N NaOH, then washed in 0.01M PBS (pH 7.0). The photocrosslinking process was accomplished by immersing SIS in 0.1% methylene blue solution for 30 min and overlapping 2 or more

sheets to expand the size, after which it was exposed to visible light (250 W) for 3 hours at room temperature. To construct a tube, 3 layers of the methylene blue treated SIS were wrapped around a 6-mm diameter plastic tube and then exposed to visible light. These ReSIS grafts were rinsed in saline for 3 days until all visible methylene blue was removed. After sterilizing the ReSIS in 75% ethanol and then rinsing it in 0.01M PBS (pH 7.0) buffer, the ReSIS was stored at 4°C in 1% Neomycin/Polymyxin B solution until used.

Surgical Techniques

Twenty female domestic pigs (13.6–18.2 kg), were studied for this project. Of these, 14 pigs underwent bladder augmentation using a sheet of ReSIS and 6 underwent subtotal ureteral replacement using tubular ReSIS. Strict adherence to animal handling procedures of the Department of Comparative Medicine at Oregon Health Science University was maintained.

All animals were sedated with an intramuscular (im) injection of 1.5 ml Telazol followed by general endotracheal anesthesia, using 1–2% Halothane inhalant. The anesthetized pig was positioned, shaved, and prepared in a sterile fashion. Heart rate and O_2 saturation were monitored during the surgery.

For the bladder repairs, a low midline incision was made in the abdomen exposing the bladder, and a 3.5 cm × 3.5 cm ellipse of anterior bladder wall was excised. This defect was then repaired using a matching ellipse of ReSIS sewn in place with a running 3–0 chromic suture. Four 5–0 Prolene sutures were placed around the ReSIS to mark its position (see Figure 1A). The abdominal incision was closed with running 0 Vicryl. An 8-Fr red rubber catheter was placed transurethrally and sutured to the perineal skin. The catheter was removed in 5 to 7 days.

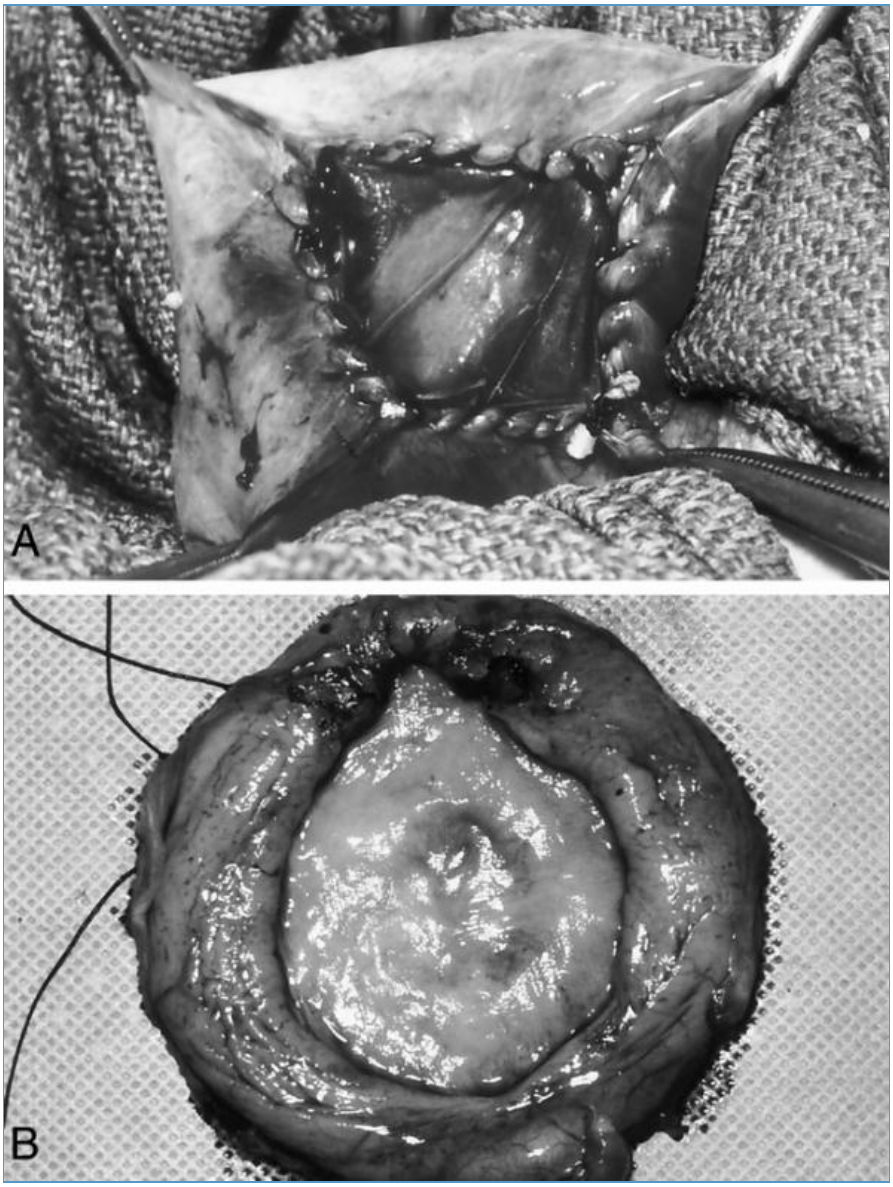

Figure 1. (A) A bladder defect was repaired by ReSIS Sheet in a porcine model. (B) Gross examination of the

bladder interior showed that the bladder defect was covered by smooth urothelium at 3 months postsurgery.

For the ureter repairs, a right paramedian retroperitoneal approach was made to expose the right ureter. A 12- to 14-cm length of ureter was mobilized, and the middle 10 cm resected. The tubular ReSIS, 8 cm in length, 6 mm in diameter, and 0.1–0.2 mm in wall thickness was then used to reconstruct the ureteral defect (see Figure 2A). Both ends were spatulated, and a running 6-0 Vicryl suture was used to effect the anastomoses. Before completion of the anastomosis, a 13-Fr silicon ureteral stent 18 cm long was placed in the ureter from the bladder to the kidney. The bladder and the abdominal incision were closed in standard fashion using a running absorbable suture after making sure there was no leakage or bleeding at the anastomotic sites. The bladder was drained with a 12-Fr urethral catheter sutured to the animal's perineal skin and cut short to allow chronic drainage. It was removed after 1 week. The ureteral stent was kept in place until the animal was euthanized.

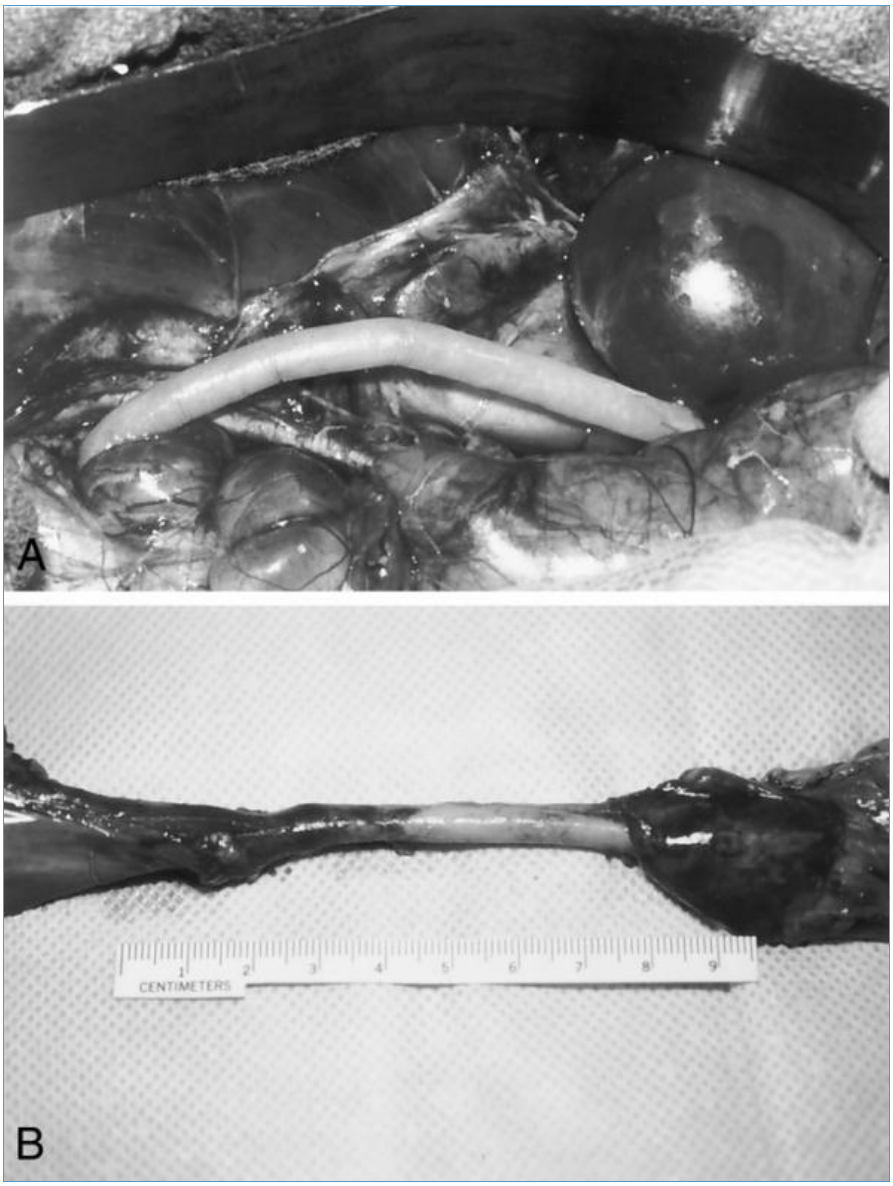

Figure 2. (A) The segmental ureter defect was replaced by tubular ReSIS. (B) Review of a gross specimen of ureter at 3 months postsurgery revealed that the defect was replaced by fibrous tissue. There were no adhesions on the substitute surface.

Animals were maintained on antibiotics for 14 days (Ampicillin and Gentamycin). Scheduled euthanization of the pigs occurred at 1, 2, 4, and 12 weeks. Before they were killed, intravenous pyelography (IVP) and antegrade pyelography were performed. The specimens were harvested for histologic evaluation.

Histologic Staining

The specimens were immediately fixed in 10% formalin and subsequently embedded in paraffin for permanent sectioning. Collagen and elastin content, calcification, and smooth muscle regeneration were analyzed using Trichrome, VVG, Von Kossa's, Actin, and hematoxylin and eosin (H & E) stains.

Results

Of the 14 animals receiving the ReSIS sheet for bladder augmentation, two were killed at 1 week, two at 2 weeks, three at 4 weeks, three at 8 weeks, and three at 12 weeks. One pig died of bowel complications.

At 1 week, gross inspection of the bladder repair with ReSIS showed an external fibrous exudate on the ReSIS. There were some adhesions on the peritoneal side of the graft between the ReSIS and omentum. Whether they represented a reaction to surgical trauma or a fusion between the ReSIS and other tissues could not be determined. Histologically, transitional urothelial cells were found lining the ReSIS by a week (see Figure 3A). By weeks 2 and 3, fibroblasts and mononuclear cells had infiltrated the ReSIS and neovascularization appeared (see Figure 3B). The urothelial lining also became more complex, containing multiple layers of transitional cells. A mild inflammatory reaction was observed surrounding the ReSIS in the first 2 weeks. Four weeks after surgery, a definite submucosa was present and the ReSIS was beginning to degrade. By 8 weeks, the ReSIS fibrils began to lose their tight formation and show obvious degradation (see Figure 3C and D). Initial muscular cell regeneration was demonstrated at 12 weeks after surgery. No obvious foreign body reaction, calcification, or sedimentation was noted at any time, and radiologic studies demonstrated normal renal function and bladder outline.

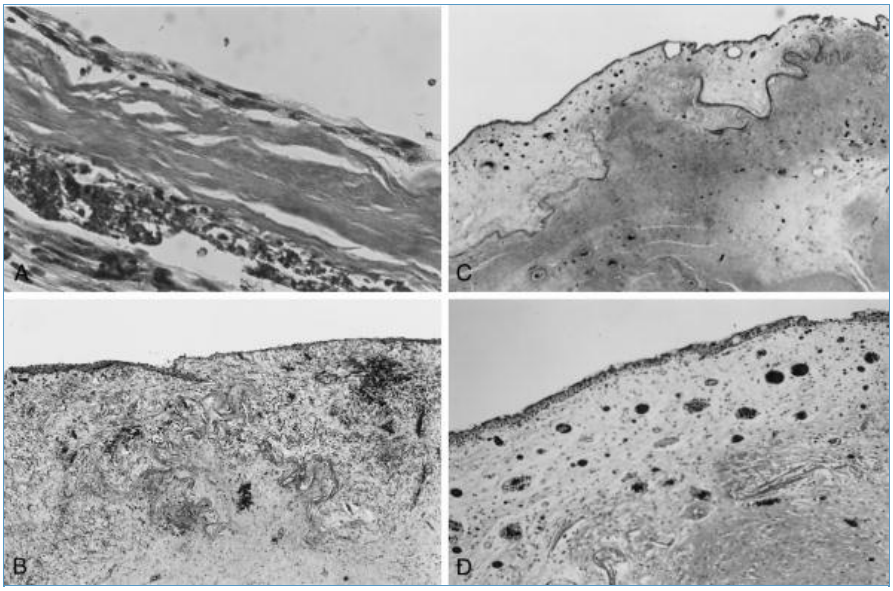

Figure 3. Histologic examination of ReSIS repaired bladder tissue (trichrome stain). (A) A transitional urothelia layer lined the ReSIS at 1 week after surgery (original magnification 400%). (B) By 2 weeks, early fibrous tissue had formed, and the ReSIS fibrils loosened. Urothelium developed multiple layers, and a mild inflammatory reaction surrounded the ReSIS at 2 weeks (original magnification, ×50). (D) After 4 weeks, a new submucosa was identified with neovascularity (original magnification 100%). (C) The ReSIS fibrils were partly degraded (original magnification 50%).

Ureteral replacement using tubular ReSIS was performed in six animals. One animal developed a urinoma at the anastomotic site, requiring premature euthanization. The five remaining animals were studied and sacrificed at 1 week (1), 2 weeks (1), 4 weeks (1), and 12 weeks (2). Antegrade pyelography demonstrated various degrees of hydronephrosis and dilatation of the proximal ureters, as well as some evidence of stenoses at the anastomotic sites. The IVP confirmed renal function with varying degrees of impairment of the ipsilateral kidneys. No calcification was seen within or around the urinary tracts of any of the animals.

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On gross inspection, the external surface of the ReSIS had no adhesions, but some fibrous scar tissue surrounding the anastomotic site was present in all animals (see Figure 2B). Histologic studies revealed the presence of some inflammatory cells infiltrating the graft at 1 to 2 weeks after implantation. By 3 weeks, transitional urothelium began to line the graft at both anastomotic ends, and capillary neovascularization could be seen in the wall of the graft. No evidence of calcification or calcium salt sedimentation was observed. At 3 months after implantation, a multilayer urothelium supported by new blood vessels within the ReSIS could be identified. Hyperplasia of the native urothelium was observed at the anastomosis. No sign of smooth muscle cell ingrowth was seen within the graft matrix, although abundant fibroblasts were present throughout in the ureteral substitutes. Antegrade pyelography again showed varying degrees of ureteral dilatation and hydronephrosis. Excretory urography suggested diminished renal function (Figure 4), and there was no evidence of peristalsis in the tubular ReSIS substitution graft.

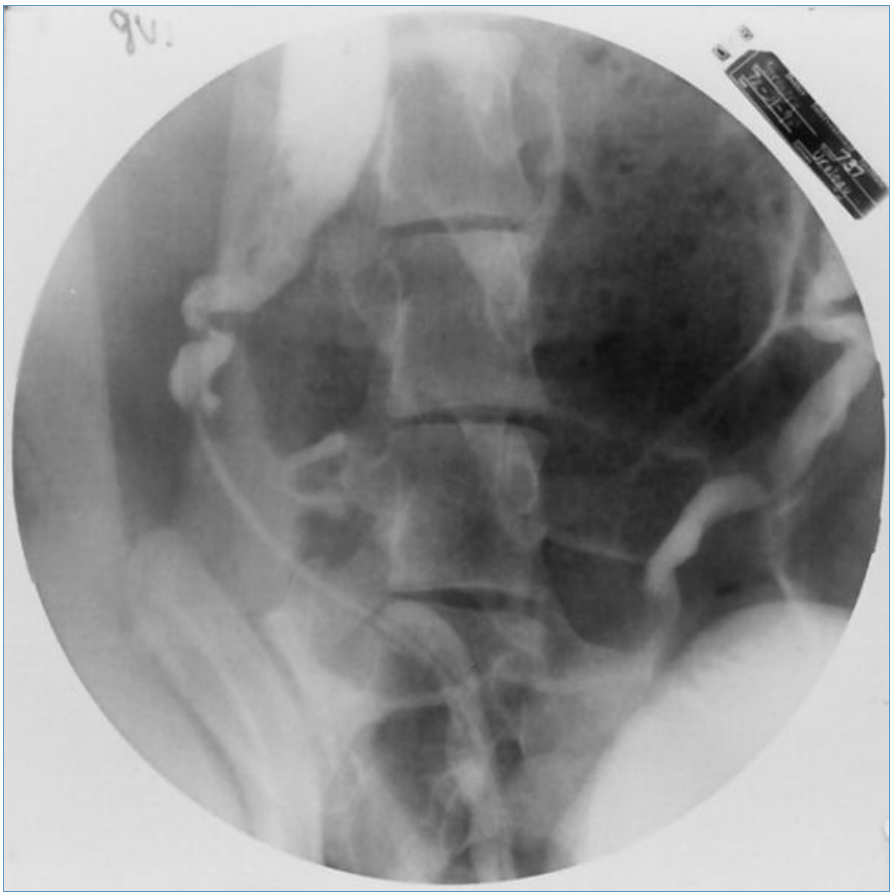

Figure 4. Excretory urography showed bilateral ureters at 2 week. A segment of the right ureter was replaced by ReSIS, and a ureteral stent was still placed.

Discussion

The ideal biomaterial for urinary tract tissue replacement would allow consistent regeneration of tissue structure and maintenance of its functions. Previous studies have achieved some success using native SIS and a bladder acellular matrix graft in animal experiments. 6,7 In these studies it appears that the grafts serve as a framework of collagen and elastin for the ingrowth of all bladder wall components. In our study, a process was created whereby SIS could be fashioned into larger sheets and tubes and thus be used for bladder or ureteral replacement. The ReSIS allowed rapid urothelial regeneration, ingrowth of new blood vessels, and the orderly deposition and organization of new collagen. The advantage of ReSIS over SIS is that larger pieces and varied shapes can be constructed with relative ease, obviating the need for multiple suture lines and, thus, the risk of urinary leakage.

After implantation the ReSIS became integrated within the host bladder by 4 weeks. Histologically, the normal components of the bladder wall were identified, while the ReSIS scaffolding showed signs of degrading. No foreign body response, calcification, or sedimentation was observed in any of the animals. By 12 weeks an intact urothelium, submucosa, and initial muscular layer had regenerated.

Previous studies have shown that SIS is a promising biomaterial for bladder augmentation and that the regenerated bladder and normal bladder have similar viscoelastic properties. 8 Our study demonstrated that the photocrosslinking technique used to form the multilayered ReSIS maintains the positive attributes of the SIS, while overcoming its size limitations. This should add to its usefulness in performing larger bladder augmentations.

When faced with severe damage to a long section of ureter, from either trauma or disease, the surgeon is faced with the task of either reconstructing the ureter or autotransplanting the kidney, both of which procedures can be complicated and time consuming. Sacrificing the kidney and relying on the function of the contralateral kidney is easier and often is the option chosen. Ureteral replacement has long been a subject of interest to urologists, and various synthetic and extract materials have been used to replace all or part of a diseased ureter. Unfortunately, these materials have uniformly failed due to bioincompatibility, the lack of peristaltic activity, and the development of salt deposits in the replaced segment. 9–14 In the present study, a ReSIS tube for ureter replacement was created by treating SIS with photocrosslinking.

A successful ureteral substitute must have the following characteristics: (1) it must act as a template for the regeneration of urinary tract tissue and cell components; (2) it must provide unobstructed drainage of urine from the kidney to the bladder; and (3) it must prevent calcification or formation of calculi on the tissue substitute. The ReSIS did allow urothelialization within its lumen and new blood vessel ingrowth into the heterograft matrix. This process began as early as 1 week postgraft, as evidenced by the histologic findings. By 3 months, mature urothelium composed of multiple cell layers and supported by an underlying vascular plexus was present. There also were abundant fibroblasts present in the heterograft matrix, although no evidence of smooth muscle regeneration was identified by 3 months. No evidence of calcification or stone formation was seen. A moderate inflammatory response in the tissue surrounding the ReSIS was seen at 1 to 2 weeks, but this inflammation decreased by 3 months. A ring of fibrotic tissue at the anastomotic sites led to various degrees of ureteral stenosis. Hyperplasia of the native urothelium was also observed at the anastomosis by 3 months.

The most challenging aspect of ureteral replacement is maintaining the normal drainage pattern of the kidney, which ideally requires the recovery of peristaltic activity throughout the entire ureter. This recovery depends upon both smooth muscle development and reinnervation of the ureter. We saw neither in this study, although smooth muscle cells may require a longer time to develop, and, certainly, reinnervation can take up to a full year to occur.

We also observed some decrease in renal function. We left ureteral stents in place throughout the study to protect the kidneys from possible obstruction due to ureteral stenoses. However, the stents also subjected the kidneys to reflux caused by the pressure of the contracting bladder during micturition. We believe that this reflux added to the hydroureteronephrosis, and that this effect was even more profound on the growing kidneys. This study suggests that the kidney needs to be protected from reflux as well as contraction or compression of the ureteral substitute.

ReSIS holds promise for being an ideal urinary tract substitute. Viscoelasticity studies still need to be performed, as do long-term studies to determine whether smooth muscle cells and innervation eventually develop and form a physiologically functioning graft. Furthermore, ReSIS needs to be studied as a xenograft if it is to have any practical application. We would expect it to have biocompatibility similar to SIS, which has been used in both rabbits and dogs. This initial study suggests that ReSIS maintained the successful characteristics of SIS while having the advantage of improved ease of use. This allows for greater versatility and application not only to large bladder augmentations and repairs but also to repair of ureteral defects.

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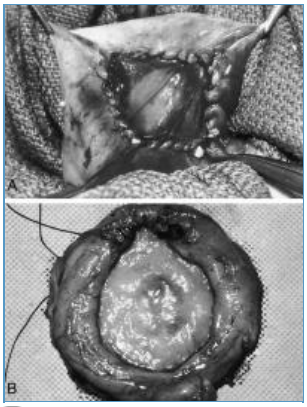
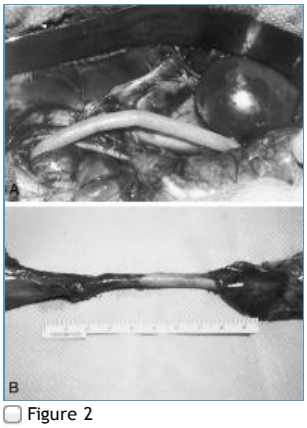


Figure 1



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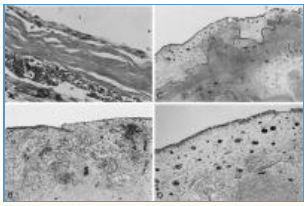

Figure 3

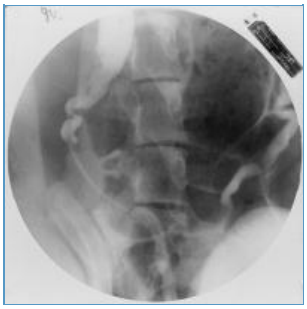

Figure 4

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