Different Outcomes in Urethral Reconstruction Using Elastin and Collagen Patches and Conduits in Rabbits

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Abstract: *Objectives:* To study the feasibility of urethral reconstruction with two urethroplasty techniques using an elastin and collagen heterograft in rabbits. *Materials and Methods:* Fifty-two male rabbits were studied. Two types of injury, (1) a 1.5×0.6 cm^2 semicircumferential defect; (2) a 1.5 cm segmental defect of the penile urethra, were created and repaired using size-matched elastin and collagen patches or tubed conduits. Urethral repair by primary closure for the type 1 injury and a tubularized autologous bladder mucosal graft for the type 2 injury served as controls. At 3 months, urethral diameter was measured with retrograde urethrography. The animals were then euthanized for histological examination. *Results:* The postoperative complication rate was significantly higher in the urethral reconstructions using tubed collagen (83%) and elastin (50%) grafts compared to the patch onlay grafts ($p = 0.001$ for collagen and $p = 0.01$ for elastin) and tubularized ABM (10%, $p = 0.003$ and 0.05, respectively). At the type 2 injury site, a dense circumferential fibrosis developed after all repairs. Only minimal ventral fibrosis presented in the type 1 injury repair. The intensity of chronic inflammation and fibrosis was greatest when collagen was used for the urethral repair. In the elastin urethral repairs the urethral diameter decreased significantly for the tubed repair compared to the patch onlay ($p = 0.02$). *Conclusion:* Urethral injury repair using elastin and collagen biomaterials is feasible in the rabbit model. The results of onlay urethroplasty using the elastin and collagen patches are significantly superior to those using the elastin and collagen tubed conduits. © 2006 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 81B: 269–273, 2007

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INTRODUCTION

Urethral re-stricture is a common postoperative complication following urethroplastic surgery, particularly in long segmental urethral reconstruction. The management of a long segmental (>4 cm) defect of anterior urethra often requires extra tissue grafting.[1] In these instances, urethral reconstruction may be accomplished in a variety of ways but typically utilizes autologous tissue such as skin, bladder, or buccal mucosa either in an onlay or tubularized fashion. The choice of urethroplasty depends on the location and severity of the urethral lesion, availability of graft tissue, and the surgeon's experience. Occasionally, it is difficult to obtain suitable tissue because of multiple prior urethroplastic surgeries, and in these instances it would be desirable to use an alternative tissue substitute such as a tissue-engineered biomaterial. In addition to shortening surgery time, using a tissue-engineered biomaterial would eliminate the morbidity of the donor site wound. Various biomaterials including heterogeneous native tissue[2–4] and synthetic polymers[5,6] have been investigated for urethral reconstruction.

In our laboratory, elastin[7] and collagen[8] based biomaterials, both derived from porcine tissue, have been investigated for tissue substitution. In this study, we investigated the feasibility of using elastin and collagen based biomaterials in onlay patch repairs and segmental tubular replacements for urethral reconstruction.

MATERIALS AND METHODS

Biomaterials

Elastin heterografts were obtained through a NaOH extraction process of porcine carotid arteries as described previously.[7] Briefly, fresh porcine carotid arteries from local abattoir were defatted and washed in 0.9% saline, then placed in 80% ethanol for 24 h at 4 °C with shaking. The carotids were rehydrated in 0.05M Hepes buffer (Sigma, MO) for 1 h and elastin matrix was extracted using 0.25M NaOH with
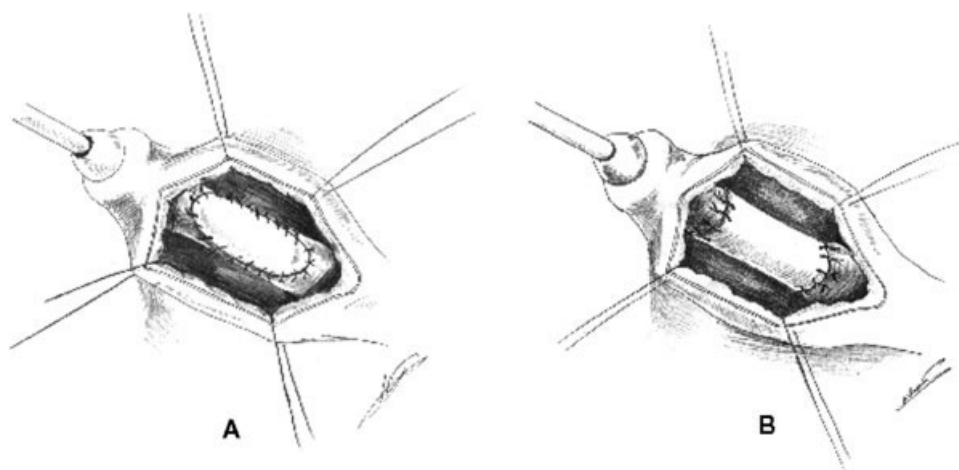
Figure 1. Diagram represents (A) type 1 injury repair with a patch graft and (B) type 2 injury repair with a conduit graft.

sonication for 70 min at room temperature. The extracted elastin appears white and translucent, histologically displays multilaminar fiber structures, is free from other extracellular matrix proteins, and has no detectable cellularity. The resultant elastin biomaterial is thermally stable to 143 °C and tolerates conventional autoclaving sterilization process. The elastin grafts were rehydrated in 0.05M Hepes buffer and then autoclaved at 115 °C for 30 min. The elastin samples were stored at 4 °C until use.

Collagen heterografts were derived from porcine intestinal submucosa.[8] Briefly, the small intestinal submucosa was decellularized in 2 mM SDS and 0.1N NaOH solution and photocrosslinked in 0.1% methylene blue with ultraviolet light (100 W) irradiation for 3 h to construct sheets or rolled to construct tubular structures. After removal of the methylene blue stain, the collagen structures were stored in 10% neomycin solution at 4 °C until use. The procedure was performed under sterile conditions.

Animals

Fifty-two male New Zealand white rabbits, weighing 3–4 kg, were divided into two groups according to the type of injury (Figure 1). In group I, a 1.5 × 0.6 cm^2 semicircumferential oval defect of the ventral penile urethra (type 1 injury) was created and repaired using a 1.6 × 0.7 cm^2 elastin ($n = 9$) or collagen ($n = 9$) patch graft, or by primary closure ($n = 8$). In group II, a 1.5 cm segment of the penile urethra was resected (type 2 injury) and replaced with an elastin ($n = 10$) or collagen tube ($n = 6$) 0.5 cm in diameter and 1.6 cm long, or an autologous bladder mucosal tube (ABM, $n = 10$) ~0.7–0.8 cm in diameter and 1.6 cm long. The animals were maintained for 3 months postoperatively.

Surgical Procedures

The protocol for the animal study strictly followed the NIH Guidelines for the Care and Use of Laboratory Animals and was approved by the *Animal Care and Use Committee of Oregon Health Sciences University* (*OHSU*). All procedures were done at the OHSU animal research facility.

The surgical procedures were performed with the animals supine and under general endotracheal anesthesia using 1–2% *Halothane* inhalant. After the animal was shaved, sterilized, and draped, an 8 Fr. urethral catheter (pediatric silicon feeding tube) was placed transurethrally into the bladder for urinary drainage. Through a ventral phallus skin incision, a 2 cm length of urethra was exposed. The injuries to the urethra were created and repaired with interrupted 6-0 Vicryl suture using one of the aforementioned techniques. In group I, urethroplasty was performed with the heterografts in an onlay fashion. Primary closure was used as the control. In group II, tubularized ABM served as the control. The urinary bladder was exposed via a midline laparotomy approach. ABM was harvested prior to urethral transection by carefully separating the seromuscular layers from the mucosa with the bladder distended. An approximate 3 × 2 cm^2 bladder mucosal graft was harvested and tubularized with 8-0 running vicryl suture. The bladder and abdominal skin were then closed in a conventional fashion. The tubularized elastin, collagen or ABM was positioned between the distal and proximal ends of the urethra and the transurethral catheter was passed through it into the bladder. End-to-end anastomoses were performed in a spatulated fashion. The phallus incision was closed in two layers with running 6-0 Vicryl. Hemostasis was obtained with a needle-tip monopolar electrocautery. Antibiotics (Baytril 5 mg/kg, SID) and analgesics (Buprenex 0.01–0.05 mg/kg, SQ) were given for 3 days postoperatively. The transurethral catheter was cut short and anchored securely to the perineal skin with sutures, and removed after 7 days.

During follow-up, the animals were watched closely for signs of postoperative complications. If there was a severe complication involving urethral stricture, bladder retention, fistula, and deverticulum confirmed by retrograde urethrography, the animal was euthanized and counted as a failure. The diseased animal was also excluded from the study. Otherwise,
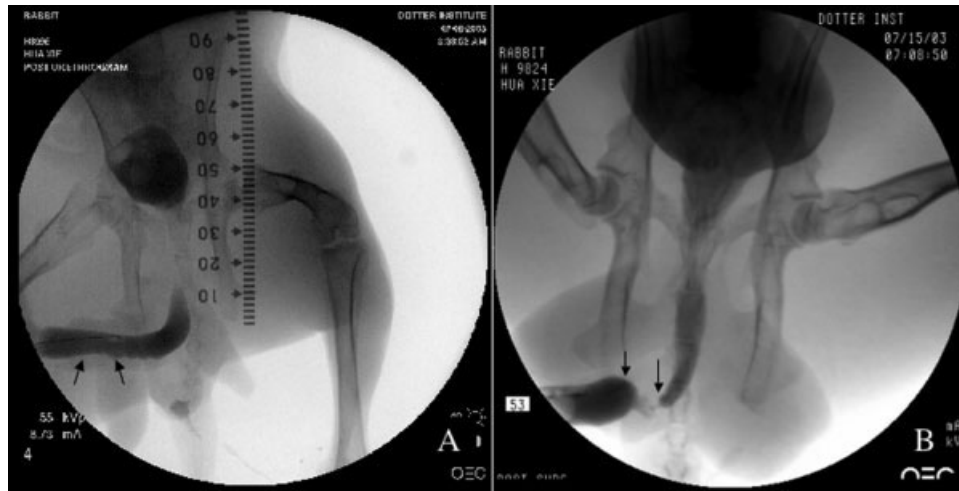
Figure 2. Postoperative urethrograms: (A) shows urethral caliber with slight stricture (between arrows) in type 1 injury repair; (B) shows a significant urethral stricture (between arrows) with urinary bladder retention in type 2 injury repair with an elastin tube.

the animal was counted as a success and sacrificed after 3 months. The urethral diameter measurement was only performed in the succeed animals. Before euthanasia, retrograde urethrography was performed to measure the diameter of the urethra at the sites of repair and normal urethra. The narrowest point of repair site and an average diameter of the normal urethra at the proximal and distal ends of the repair were recorded to determine the percent stricture. The penile specimen was examined grossly to record the size of repaired area for tissue shrinkage and then prepared for histological examination.

Statistic Analysis

The percentage of remaining urethral diameter at repaired site among different repair techniques was analyzed by two-tailed Student's t test. χ^2-test was used to analyze the differences of proportions of success or failure among grafts and surgical methods. p values <0.05 were considered statistically significant.

RESULTS

All animals tolerated the initial surgery. In the type 1 injury repair, all animals survived without any sign of difficulty urinating or urinary retention. There was mild urethral stricture formation in the elastin and collagen onlay repairs as well as the repairs using the primary closure [Figure 2(A)]. The urethral diameter remained 84% ± 8% in elastin, 87% ± 13% in collagen, and 71% ± 13% in primary closure repairs. In the type 2 injury repairs, five animals (5/10, 50%) using elastin tube developed postoperative complications, which included severe urethral stricture ($n = 3$), fistula ($n = 1$), and acute pyelonephritis ($n = 1$). The rest of the animals in this group survived with 60% ± 16% of remaining urethral diameter. Urethral diameter decreased significantly in the elastin tube repair compared to the elastin patch onlay (84% vs. 60%, $p = 0.02$). Most animals (5/6, 83%) with collagen tube repairs

developed severe stricture that were sacrificed prior to end point of the study [Figure 2(B)]. Only one animal survived with ∼50% of remaining urethral diameter in this group. In the urethral repair with tubed ABM, a postoperative urethral diverticulum (1/10, 10%) at the repair site was verified by a retrograde urethrogram. The nine other animals survived with 66% ± 17% remaining urethral diameter (Figure 3). The postoperative complication rate was significantly higher in type 2 injury repairs using collagen and elastin conduits than in the tubed ABM repairs ($p = 0.003$ and 0.05, respectively). Comparing the urethral repairs between the injury types, the postoperative complications increased significantly in the type
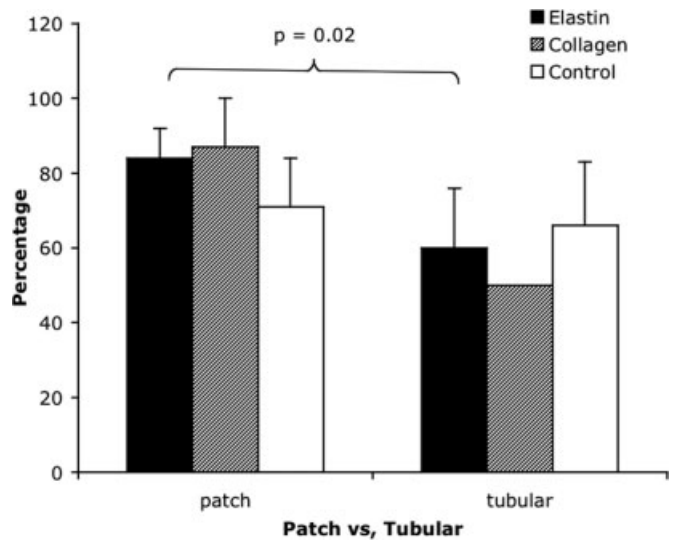
Figure 3. Comparison of the percentage of remaining urethral diameter (mean ± SD) after urethroplasty using elastin, collagen, and controls in 3 months survived animals. A significant difference was found between elastin patch and tube repairs. Only one case is presented in collagen tubular repair. Five out of six animals in this group developed severe urethral stricture that were euthanized prior to our study end point.
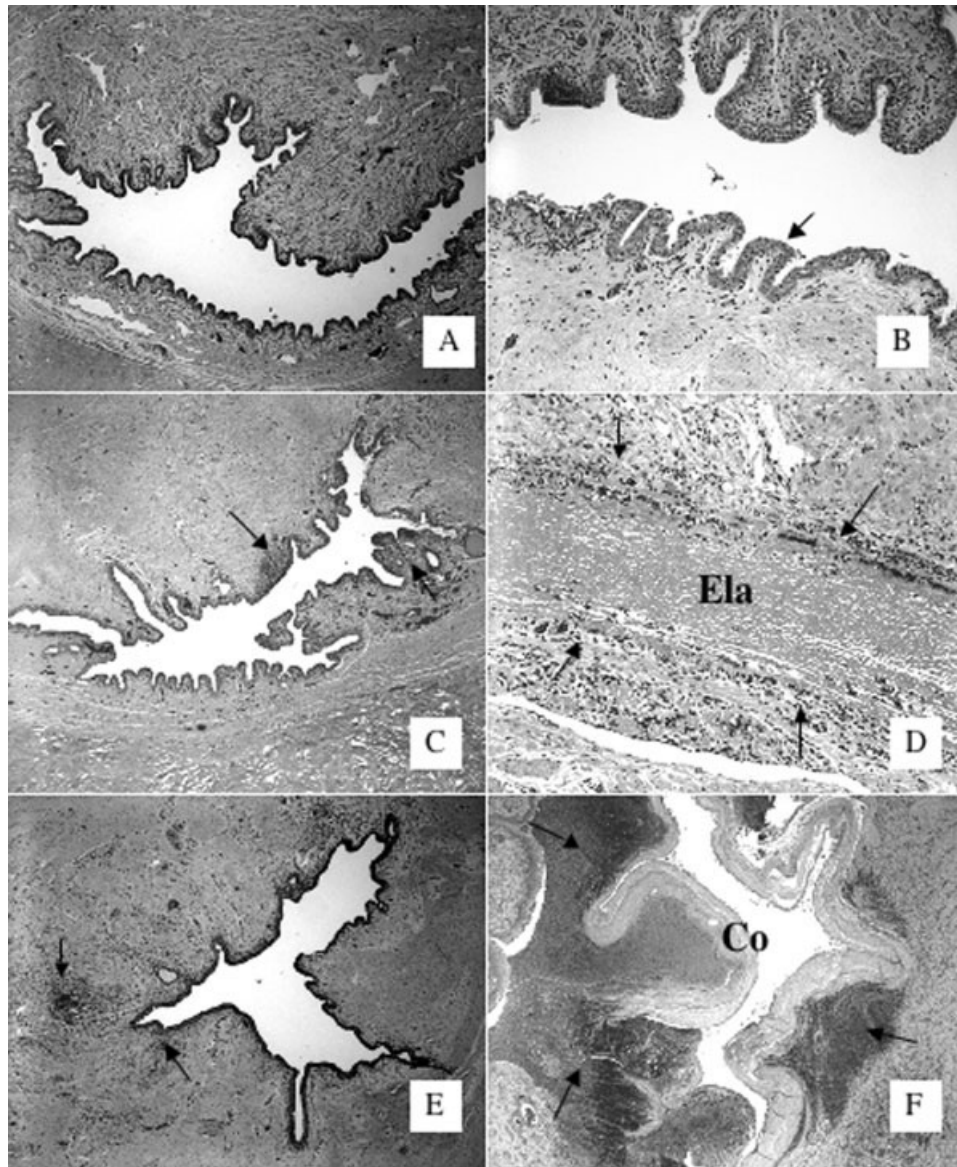
Figure 4. Histological examination (H&E stain): (A) transversal section of normal rabbit penis (×50); (B) proximal junction of urethral end and ABM graft with normal urothelium lining and local fibrosis reaction (bottom, ×100); (C) transversal section of neo-urethra shows chronic inflammation foci surrounding urethral lining (arrows) 3 months after elastin tubular graft repair (×50); (D) elastin graft (Ela) surrounded by inflammatory cells at 3 weeks (×100); (E) transversal section of the neo-urethral from collagen tube repair shows a dense fibrosis with sporadic chronic inflammation (arrows) surrounding the urethra (×50); (F) intensive inflammatory response around extruded collagen (Co) tubular graft at 3 weeks, the sample was from a failed study (×100).

2 injury repairs using elastin (50% *vs*. 0%, $p = 0.01$) and collagen (83% *vs*. 0%, $p = 0.001$).

Histologically, the type 1 injury repairs with elastin, collagen, and primary closure had similar findings at the repair sites. After 3 months, all sites demonstrated complete re-epithelialization of the urethral lumen, minimal fibrotic scarring in the ventral aspect of the urethra, and no appreciable inflammation, calcification or remnant of the implanted material. In the type 2 injury repairs, 3 months after surgery there was intact urothelial regeneration with a dense, circumferential periurethral fibrosis at the graft sites regardless of the type of graft used. The amount of chronic inflammation and fibrosis was greatest, however, in the tubularized collagen repair (Figure 4). No graft remnants or calcifications were found at any of the successful tube graft sites. In the urethral repair with tubed ABM, a mild urethritis cystica was noted at the repair site.

DISCUSSION

Urethroplastic technique for repair of urethral injuries varies depending on the location and severity of the urethral lesion as well as the preference of the surgeon. Primary end-to-end

anastomoses have the best outcomes for the treatment of short urethral defects in terms of complications and re-stricture rates after urethroplasty. For longer and more complicated reconstructions, however, grafts are necessary, especially when local skin flaps are not available. In these cases, extra genital skin, bladder mucosa, and more recently buccal mucosa have been used to affect the repair. Andrich and Mundy reported that the re-stricture rate in 128 patients who underwent urethroplasty with buccal mucosa was 11% in patch grafts and 45% in tubed grafts.[9] Other noteworthy experimental work from El-sherbiny compared the efficacy of using skin, buccal mucosa, and bladder mucosa in both tubed and onlay grafts to repair urethral defects in a canine model.[10] Their results demonstrated a greater stricture rate with tubularized grafts (66%), which included 4/4 (100%) using skin, 3/4 (75%) using bladder mucosa, and 1/4 (25%) using buccal mucosa. Interestingly, a different result was reported by Metro et al.[11] who found that patients with onlay grafts had a higher subsequent stricture rate than those treated with a tubularized graft.

In this study, two tissue-engineered biomaterials, elastin- and collagen-based, have been tested as urethral grafts for the reconstruction of urethral defects. The advantages of using tissue-engineered rather than autologous grafts lie in the elimination of the graft retrieval process, thereby shortening surgical and hospital time, and eliminating the associated morbidity of a donor site. These biomaterials have been evaluated for duodenal,[7] bladder, and ureteral[8] injury repair in our laboratory. The results indicated that the materials are biocompatible with excellent tissue regeneration proprieties. Our current study confirmed this conclusion for the elastin and collagen biomaterial as an onlay graft for urethral injury repair. However, when used in a tubularized form to replace a segment of urethra, these grafts were associated with a significantly higher complication rate compared to both the onlay grafts and the tubularized ABM graft. The higher complication rate seen with reconstruction of entire segments of urethra *versus* partial circumferential urethral defects both for flaps and grafts is generally recognized.[1] Given both reported and personal experience with collagen-based biomaterials,[8,12] however, it is difficult to explain the high rate of stricture and adjacent inflammation seen with the collagen graft compared to both the elastin and the ABM tubularized grafts. A possible explanation for this difference is that the ABM tubed grafts were larger than the elastin or collagen conduits. Typically, urethroplasty grafts are over-sized to allow for a 15–25% graft contraction during wound healing.[1] In the type 1 injury repair, we over-sized our grafts by $\sim 20\%$. In the type 2 injury repair, the diameter of the elastin and collagen tube graft was fixed, but the ABM tubularized graft was fashioned with at least 25% excess material. The presence of perigraft inflammation in all the tubularized grafts was noted and, not surprisingly, was least intense in the ABM graft. This may be due to our processing technique of both elastin and collagen and possible cross-species antigenic remnants in these xenogenic grafts. In those successful repairs with tubularized grafts, the urethral diameter remained an average of 60 and 66% in the elastin and ABM group, respectively, without signs of urinary retention or urinating difficulty. Clinically, there is no quantitative method to determine the degree of urethral stricture. In this study, normal urinary function was seen in urethral diameters ranging from 40 to 100% of native urethral diameters.

We conclude that the elastin and collagen biomaterials can be used successfully as grafts for onlay urethroplasty repairs in the rabbit model, but for segmental urethral defects, these biomaterials are associated with a significant postoperative complication rate when compared to the use of tubularized autologous bladder mucosa. The reason for this difference is not clear and needs to be further evaluated.

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