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Decellularized Porcine-Derived Blood Vessel Matrix Graft for Urethral Replacement in a Rabbit Model

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ABSTRACT

Objective: To evaluate a xenographic urethral replacement model utilizing porcine-derived, decellularized blood vessel matrices in rabbits.

Materials and Methods: In 17 male rabbits, a 1 cm tubular segment of porcine, acellular blood vessel matrix replaced a 1 cm urethral defect without a postoperative catheter. The animals were sacrificed at varying intervals (1, 3, and 6 months) and assessed for graft patency and integration properties.

Results: All but 1 animal survived. One animal died of unknown etiology 1 month after surgery. In all 17 rabbits, the urethra was patent, without evidence of stricture formation as confirmed by gross inspection and passage of a 10 Fr catheter at the time of euthanasia/tissue harvest. At 1 month, histological examination revealed epithelialization, host-cell infiltration, angiogenesis, and migration of the smooth muscle. The smooth muscle bundles were more organized by 6 months. No significant fibrosis or stricture was observed in the anastomotic area.

Conclusion: This successful experiment would support efforts for further investigation of a potentially off-the-shelf product using acellular blood vessel matrices for single-stage urethral reconstruction without requiring stem cell technology. To our knowledge, this is the first report of a xenograft blood vessel matrix for urethral substitution.

INTRODUCTION

Definitive surgical repair for urethral stricture disease is often complex and sometimes requires a staged approach with tissue transfers and/or grafting. Reported donor sites include grafts from skin, bladder, tunica vaginalis, peritoneum, rectum, and, most commonly, buccal mucosa. Harvesting from each of these donor sites is associated with morbidity, extended operative times, additional procedures, and prolonged hospitalization. In addition, donor graft sites may be limited in size and, in some cases, unavailable due to previous surgery, malformation, or disease.

KEYWORDS: Urethra, graft, repair, urethroplasty, biomaterial

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Figure 1. Intraoperative photo of BVMx graft in place.
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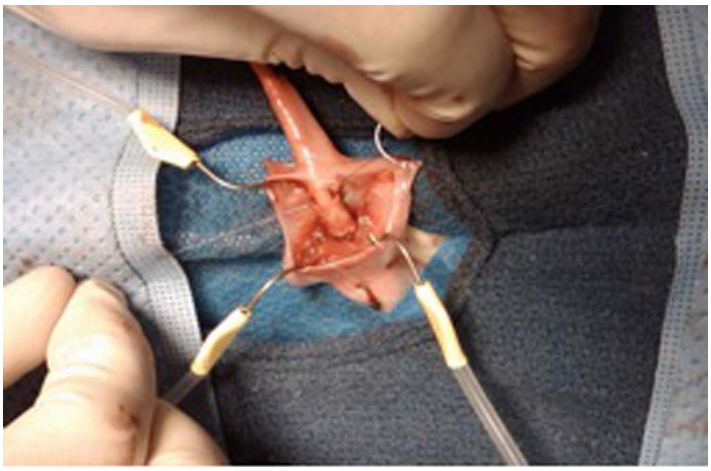
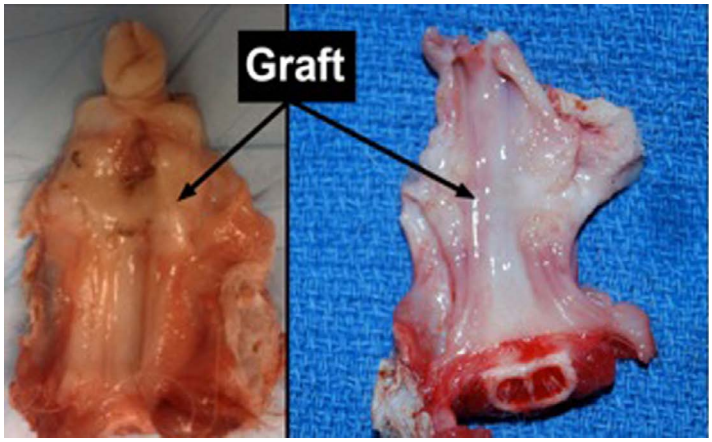


Figure 2. Left: Graft/native urethra interface at 1 month. Right: Graft/native urethra interface at 6 months.
http://dx.doi.org/10.3834/uij.1944-5784.2012.04.08f2



An ideal substitute for urethral replacement would be an off-the-shelf material that is readily available and could be used without complex systems of storage and cell seeding. It would maintain patency as a tubular construct, be easy to handle, incorporate host tissue, allow neovasculature, and undergo minimal retraction and degradation.

Our study evaluated a xenograft blood vessel matrix for urethral replacement in a rabbit model.

MATERIALS AND METHODS

Seventeen New Zealand white male rabbits, weighing approximately 3 kg, were supplied with a liquid diet and unlimited water for 72 hours prior to the operation, and then water for 24 hours before surgery. Antibiotic prophylaxis was administered preoperatively. Rabbits were preanesthetized with an intramuscular injection of a ketamine cocktail (100 mg ketamine/10 mg xylazine/1 mg acepromazine per ml) and anesthetized with 1 to 2% isoflurane. The perineum of the animal was prepped and draped in a sterile fashion. The urethra was exposed, incised, and retracted with a retractor. A 1 cm segment of penile urethra was excised and a 1 cm by 0.5 cm tubularized blood vessel matrix (BVMx) xenograft segment was placed in the urethral defect. The unstented repair was performed using 6-0 Biosyn suture in a continuous fashion (Figure 1). The BVMx from porcine arteries was prepared as

Figure 3. Rabbit 11 matrix/native urethra interface at 3 months (200x). Circles: neovasculature; red: new ingrowth; green: acellular matrix; blue: native urethra.
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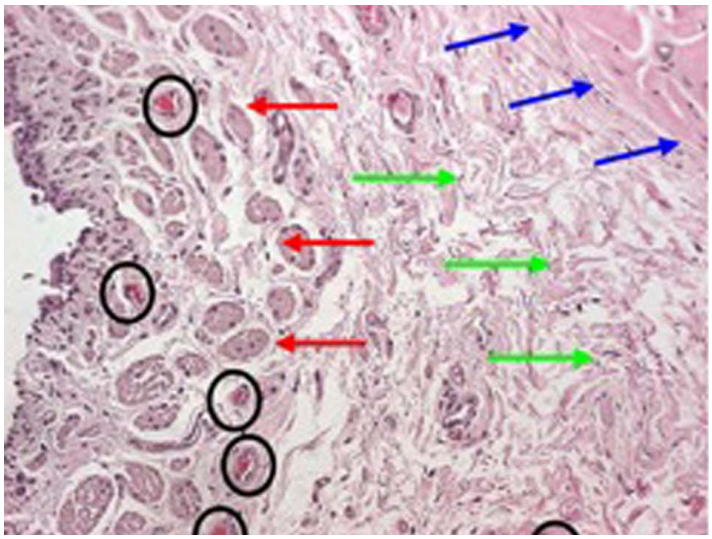

reported by Amiel et al. [3,4].

Post surgically, the rabbits were monitored by animal facility technicians, set on heating pads, and given subcutaneous injections of 0.20 mg/kg meloxicam daily, as needed, for postoperative pain relief. The investigators evaluated the rabbits, postoperatively, at day 2, and at 1, 2, 6, and 12 weeks. They checked the incision site for infection or breakdown. The rabbits were sacrificed at 1, 3, and 6 months after surgery (5 at each period, 2 for evaluation of technique). The urethra and surrounding tissue were removed, en bloc, and examined, passing a 10 Fr catheter to evaluate patency of the graft and urethra (Figure 2).

Each urethral segment was evaluated for histology, immunohistochemistry, and mechanical testing. Graft integrity, vascularity, host inflammatory response, and host tissue incorporation were examined. Sections were viewed at magnifications of 40x, 100x, and 400x, and digital images were captured using Olympus Provis AX-70 epifluorescence microscopy with appropriate filters and a cooled, charge-coupled device (CCD) camera. A minimum of 5 fields at 100x magnification were examined, and the number of polymorphonuclear leukocytes or neutrophils, mononuclear cells, and blood vessels per field were quantitated and separately scored as described by Vorotnikova et al. [5]. In addition, connective tissue deposition and organization (oriented bands of fibrous connective tissue) were semi-quantitatively categorized as 1 (totally disorganized), 2 (slightly organized), 3 (moderately organized), and 4 (well organized) (Figure 3).

RESULTS

All but 1 animal survived during our study. The cause of death at 1 month was of unknown cause, despite necropsy. Passage of a 10 Fr catheter at the time of euthanasia confirmed patency of all grafts and wide urethral caliber with no gross evidence of stricture. Gross examination at harvest showed normal urethral tissue with no evidence of scars, fistula, or retraction (Figure 2).

Light microscopy confirmed an acellular graft with an intact framework of elastin and collagen fibers prior to implantation. After harvest, the matrices contained host-cell infiltration and angiogenesis at all time points. At 1 month, a complete transitional cell layer was covering the matrix and was present throughout (Figure 3). Unorganized muscle fibers could be seen at 1 month, with an increasing number of organized muscle bundles at 3 and 6 months. The number and caliber of blood vessel lumens increased at 3 and 6 months, although sufficient for survival at the 1-month point. The ingrowth of tissue, muscle fibers, and blood vessels could be seen from all sides of the graft. Gross measurement of the graft at retrieval showed little evidence of contracture or narrowing.

DISCUSSION

Previous studies have shown that an acellular matrix is biocompatible and able to incorporate host tissue. Parnigotto et al. repaired a urethral defect in rabbits with an acellular seeded rabbit aortic matrix and showed success [6]. Our work shows the success of a xenograft blood vessel matrix for urethral replacement. Fu et al. showed that a tubularized urethral segment replaced in a rabbit model was better with a seeded matrix [7]. Our unseeded, acellular, and unstented tubular construct proves biocompatible in an animal model and able to incorporate host tissue as early as 1 month. All rabbits had a wide-caliber urethra by gross and histological inspection, without evidence of stricture, fistula, or retraction.

Because there is no ideal urethral replacement construct, a variety of experiments have been attempted [8,9]. These include the work of Chen et al., utilizing cadaveric human bladder tissue in human subjects with urethral strictures in an onlay fashion. Our study shows success with a xenograft tubular construct that is a potential off-the-shelf product. However, further studies are warranted to establish the validity of our replacement model as it applies to humans who often have longer defects. Previous studies (Xie et al., Chen et al.) [10,11] have used an onlay procedure showing similar tissue incorporation. Similarly, our tubular construct remained patent, avoided contracture, and allowed for similar tissue ingrowth and neovascularization.

This experiment provides support for an off-the-shelf product that alleviates the need for separate procedures and associated morbidity. Blood vessel matrices have excellent handling properties, are easily processed, and are readily available in a range of dimensions.

CONCLUSIONS

This successful experiment supports efforts for further investigation of a potentially off-the-shelf product using a porcine-derived acellular blood vessel matrix for single-stage urethral reconstruction without requiring stem cell technology. To our knowledge, this is the first report of a xenograft blood vessel matrix for urethral substitution.

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