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Repair of injured urethras with silk fibroin scaffolds in a rabbit model of onlay urethroplasty

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ABSTRACT

Background: Preclinical validation of scaffold-based technologies in animal models of urethral disease is desired to assess wound healing efficacy in scenarios that mimic the target patient population. This study investigates the feasibility of bilayer silk fibroin (BLSF) scaffolds for the repair of previously damaged urethras in a rabbit model of onlay urethroplasty.

Materials and methods: A focal, partial thickness urethral injury was created in adult male rabbits ($n = 12$) via electrocoagulation and then onlay urethroplasty with 50 mm^2 BLSF grafts was carried out 2 wk after injury. Animals were randomly divided into three experimental groups and harvested at 2 wk after electrocoagulation ($n = 3$), and 1 ($n = 3$) or 3 ($n = 6$) months after scaffold implantation. Outcome analyses were performed preoperatively and at 2 wk after injury in all groups as well as at 1 or 3 mo after scaffold grafting and included urethroscopy, retrograde urethrography (RUG), and histological and immunohistochemical analyses.

Results: At 2 wk after electrocoagulation, urethroscopic and RUG evaluations confirmed urethral stricture formation in 92% ($n = 11/12$) of rabbits. Gross tissue assessments at 1 ($n = 3$) and 3 ($n = 6$) mo after onlay urethroplasty revealed host tissue ingrowth covering the entire implant site. At 3 mo post-op, RUG analyses of repaired urethral segments demonstrated a 39% reduction in urethral stenosis detected following electrocoagulation injury. Histological and immunohistochemical analyses revealed the formation of innervated, vascularized neotissues with α-smooth muscle actin+ and SM22α+ smooth muscle bundles and pan-cytokeratin + epithelium at graft sites.

Conclusions: These results demonstrate the feasibility of BLSF matrices to support the repair of previously damaged urethral tissues.

Introduction

Pediatric and adult disorders of the urethra including hypospadias, trauma, and stricture disease represent significant health care burdens, which require surgical intervention to replace developmentally absent or damaged tissue to preserve urinary tract function.[1,2] Autologous tissue grafts derived from extragenital skin flaps or buccal mucosa are primarily utilized to restore urethral continuity in cases where end-to-end anastomosis is not feasible.[3,4] However, these

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approaches have been associated with adverse side effects including stricture recurrence and diverticula and fistula formation with total complication rates of 5%-54%.[5-8] Moreover, the harvesting of autologous tissues requires secondary surgical procedures, is associated with morbidity at the donor site, and donor tissue volume is often limited for reconstructive procedures.[9] These issues highlight the critical need for the development of alternative strategies for urethral repair.

Bilayer silk fibroin (BLSF) matrices represent emerging, biodegradable platforms for urethroplasty. The multifunctional graft configuration facilitates initial defect consolidation and maintenance of urethral integrity via a fluid-tight film layer, whereas host tissue integration is promoted via a porous foam compartment.[10,11] A previous study from our laboratory has demonstrated the feasibility of these grafts for onlay urethroplasty in a nondiseased, rabbit model of acute trauma wherein urethral reconstruction was performed immediately following creation of a full-thickness defect.[11] In this setting, BLSF scaffolds promoted constructive remodeling of urethral defects with neotissues capable of supporting micturition and normal urethral continuity. Parallel evaluations with conventional small intestinal submucosa (SIS) scaffolds revealed that BLSF matrices displayed superior biocompatibility with significantly less chronic inflammatory reactions at implantation sites.

Validation of biomaterial technologies in diseased animal models that mimic underlying patient pathology is necessary to accurately evaluate graft potential before clinical translation. Alterations in the regenerative capacity of host tissues can occur as a function of disease or past injury and can ultimately influence implant functional performance.[12-14] In particular, onlay urethroplasty of damaged rabbit urethras with SIS grafts showed delayed epithelialization and abnormal distribution of smooth muscle tissue in comparison to the results achieved in healthy cohorts.[13] In addition, patients who have undergone two or more failed urethroplasties and have comorbidities associated with urethral stricture disease present with an increased risk of repeat urethroplasty failure.[15] Therefore, the purpose of this study was to evaluate the ability of BLSF scaffolds to mediate tissue regeneration in urethras previously subjected to surgical injury in a rabbit model of onlay urethroplasty.

Materials and methods

Biomaterials

BLSF grafts were fabricated from aqueous silk fibroin solutions derived from *Bombyx mori* silkworm cocoons using a solvent-casting/salt-leaching protocol in combination with silk fibroin film casting as previously described.[10] The structural and tensile properties of the scaffold have been reported in published reports.[10] Biomaterials were autoclaved sterilized before surgical manipulations.

Surgical procedures

All animal studies followed guidelines prescribed by the National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals and were approved by the Boston Children's Hospital Animal Care and Use Committee before experimentation and performed under protocol 15-06-2966R.

A focal, partial thickness urethral defect was induced in adult male New Zealand white rabbits ($n = 12$, ~3-3.5 kg; Millbrook Breeding Labs, Amherst, MA) using a previously described electrocoagulation injury model.[16] Adult male rabbits were used in these studies because their anatomy permits transurethral instrumentation with a standard urethroscope.[17] In addition, the urethra of male rabbits histologically resembles the human male with a stratified epithelium supported by vascularized spongious tissue.[17] Female rabbits were excluded from these investigations because of their short urethral length for reconstructive procedures. Under isoflurane inhalation and following intramuscular injection of 0.04-mg/kg glycopyrrolate, animals were placed in the supine position and genitalia were scrubbed with a povidone-iodine solution. Rabbits were kept under mechanical ventilation for the duration of the operative procedures. Animals were first subjected to imaging evaluations (retrograde urethrography [RUG] and urethroscopy) to confirm normal anatomy. Next, a 2- to 3-mm-wide electrocoagulation injury (1 cm in length) in the anterior urethral spongiosum was performed from the 3-9 o'clock position ~2 cm proximal to the external meatus using an electrosurgical loop from a pediatric resectoscope under direct visualization with a urethroscope. Following urethral injury, free urine drainage via catheterization was maintained for 1 wk post-op with an eight French Zaontz urethral stent; afterward, animals were allowed to voluntary void. Two weeks after injury, imaging evaluations were carried out and rabbits were either euthanized for baseline histological and immunohistochemical (IHC) analyses ($n = 3$) or subjected to BLSF scaffold implantation ($n = 9$). A nonsurgical control (NSC) group of three rabbits was evaluated in parallel for all outcome analyses.

Ventral onlay urethroplasty with BLSF scaffolds was performed as previously described.[11] Following urethral catheterization, the penile urethra was sterilely exposed through a ventral midline penile skin incision and mobilized from the underlying corpora cavernosa under isoflurane anesthesia. A 5×10 mm (length $\times$ width) elliptical defect was created at the original injury site via excision of ventral urethral tissue with the penis on partial stretch. A BLSF graft of equal size was anastomosed to the defect using interrupted 6-0 polyglactin sutures. Nonabsorbable 6-0 polypropylene sutures were positioned at the proximal, distal, and lateral boundaries of the implant perimeter for identification of graft borders. Running sutures were used to close skin incisions. Rabbits were administered prophylactic antibiotics (5 mg/kg/d Baytril) for 3 d post-op, and catheterization with an eight French Zaontz urethral stent was maintained for a 7-d period after which voluntary voiding was permitted. Animals were harvested at 1 ($n = 3$) or 3 ($n = 6$) mo after repair for study endpoint evaluations.

RUG and urethroscopy

RUG and urethroscopic analyses were carried out in NSCs and in experimental animals before injury, 2 wk after injury, as well as 1 and 3 mo after scaffold implantation to assess organ