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Autologous granulation tissue tubes for replacement of urethral defects: An experimental study in male rabbits

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Summary

Introduction
Tubularized urethroplasty is commonly performed in clinical practice using genital skin flaps, bladder mucosa, and buccal mucosa. However, the long-term effects are not satisfying, and donor site morbidities remain a problem. Besides, those grafts are unavailable with malignant conditions of the urinary tract, a history of lichen sclerosis, or oral disease.

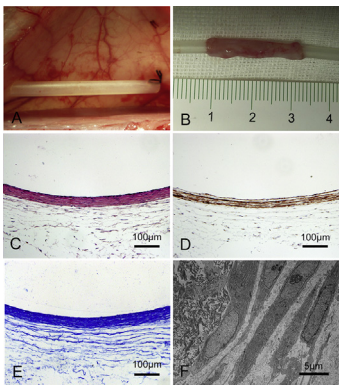
Objective
An autologous granulation tissue tube of any required length and diameter can be produced by implanting foreign objects subcutaneously (Summary Fig.). The current study aimed to investigate to what extent of length this fully autologous tissue could be used for tubularized urethroplasty, satisfying urethral patency and tissue regeneration, in male rabbits.

Study design
Twenty-seven New Zealand male rabbits were randomly divided into three groups. Silastic tubes were implanted subcutaneously in Group 1 and Group 2. By 2 weeks the granulation tissue encapsulating the tubes was harvested. In Group 1, pendulous urethral segments of 1 cm were excised, and urethroplasty was performed with the granulation tissue tube in an end-to-end fashion. In Group 2, a pendulous urethral segment of 1.5 cm was replaced with the tissue tube. In Group 3, a pendulous urethral defect of 1 cm was repaired by re-anastomosis as control. Serial urethrograms were performed at 1, 2 and 6 months postoperatively. Meanwhile, the neo-urethra were harvested and analyzed grossly and histologically.

Results
The urethrograms showed that all animals in Group 1 maintained a wide urethral caliber. In contrast, animals in Group 2 and Group 3 developed progressive strictures. Histologically, an intact urothelium with one to two cell layers lined the graft by 1 month, which was surrounded by increasing organized smooth muscle in Group 1. By 6 months, the grafts were completely integrated into native urethra. Nevertheless, extensive fibrosis occurred in Group 2 and Group 3.

Discussion
The tissue successfully maintained patency and guided urethral regeneration across a distance of 1 cm. As an epithelium-free graft, the tissue showed better results than acellular matrix for tubularized urethroplasty compared with previous studies. Nevertheless, several limitations existed: (1) the urethral defect was created in healthy urethra, which could not fully simulate the clinical situation; (2) as a small animal model, rabbit was less informative for clinical problems; (3) the tissue was inadequate for long segmental urethral replacement. Further study is needed before the procedure is used clinically.

Conclusion
An autologous granulation tissue tube grown subcutaneously could be successfully used to repair urethral defects of 1 cm in male rabbits.



Summary Figure Autologous granulation tissue tube formation. (A) Silastic tube implantation; (B) harvested granulation tissue tube 2 weeks after implantation; (C) hematoxylin/eosin staining (200×); (D) immunohistochemistry with α-SMA (200×); (E) Masson's trichrome staining (200×); (F) transmission electron micrographs showing myofibroblasts (5000×).

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Introduction

Reconstruction of the urethra for treatment of congenital defects and traumatic strictures is still a great challenge for urologists. Tubularized urethroplasty is usually required, especially for hypospadias and long segmental strictures. This procedure involves total correction of penile curvature or resection of unhealthy urethral tissues followed by bridging the urethral gap using substitute tissue. In clinical practice, such repair is commonly performed by tubularizing genital skin flaps, and bladder or buccal mucosa [1—3]; however, it causes injury to normal healthy tissue and may lead to donor site morbidities. For buccal mucosa, intraoperative hemorrhaging, damage to salivary ducts, pain, swelling, altered sensation, infection, limited oral opening and deformity have been reported [1,4—6]. Hair growth and meatal stenosis have limited the use of skin flaps and bladder mucosa, respectively [7,8]. Besides, those grafts are unavailable with malignant conditions of the urinary tract, a history of lichen sclerosis, or oral disease. Furthermore, tubularizing the graft around a catheter is time consuming, and it has always been a concern that the presence of a longitudinal suture line may potentially increase formation of a urethrocutaneous fistula [9].

A natural tubular graft is supposed to simplify the procedure and accelerate tissue healing. Previous studies have confirmed that an autologous granulation tissue tube could be formed by implanting silicone mandril into the subcutaneous tissue of animals [10]. By changing the size of the molding, grafts of different diameter and length could be produced. Such tissue demonstrated good mechanical property and biocompatibility when used to repair artery defects in a previous study [10]. The present study wanted to investigate to what extent of length this fully autologous tissue could be used for tubularized urethroplasty, satisfying urethral patency and tissue regeneration, in male rabbits.

Materials and methods

Subcutaneous tubing implantation and harvest

All animal experimentation was approved by Animal Care and Use Committee of Shandong University (Shandong, China). The rabbits were kept in an air-conditioned animal facility with 12: 12 h light: dark cycles, and free access to food and water. General anesthesia was applied by intravenous injection of pentobarbital sodium (30 mg/kg) via ear vein. Animals were placed on the operation table in supine position. The ventral abdominal wall was shaved and disinfected with 75% alcohol. A small ventral midline incision was made on the skin of 18 male New Zealand white rabbits aged 6—8 months. The skin bilateral to the incision was gently separated from the subcutaneous tissue using surgical scissors. Considering the diameter of a male rabbit urethra, 10-Fr silastic tubes (Chensheng, Shandong, China) with an external diameter of 10/3 mm (≈3.33 mm) were chosen as molding. Four 3-cm-long tubes were implanted for each animal. Tubes were placed inside the subcutaneous tissue and sutured to the dermis at one end with 6/0 polypropylene (Prolene) for fixation. The incision was closed with 3/0 polyglactin 910 (Vicryl). Penicillin G sodium (10^5 u/day intramuscular) was administered for 3 days postoperatively.

Two weeks after implantation, animals were re-anesthetized and the original incision was reopened for harvesting of the granulation tissue, which had encapsulated the silastic tubes. The silastic tube was slipped out of the tissue and discarded. Due to higher suture holding strength, the tissue with a thick wall was chosen for urethral reconstruction, and the rest for histological and biomechanical analysis. Transverse sections of the tissue tube were prepared for hematoxylin-eosin staining (H&E), immunohistochemistry analysis, and Masson's trichrome. Myofibroblast cells were stained with α-smooth muscle actin antibodies (α-SMA) (Abcam, Cambridge, UK).

Transmission electron microscopy of the granulation tissue tube

After the molding was removed, the granulation tissue tube was cut longitudinally and spread out. It was then trimmed to 1 mm × 1 mm × 3 mm, fixed immediately with 3% glutaraldehyde and postfixed with 1% osmium tetraoxide, which was followed by en bloc staining with 0.5% uranyl acetate and embedding in Epon 812. The prepared specimen was then cut into thin sections, stained, and examined under a JEOL-1200EX microscope (JEOL, Japan).

Urethral surgery and postoperative evaluation

A total of 27 male rabbits were randomly divided into three groups. The perineum and genitals were shaved and scrubbed with povidone-iodine solution. After being separated circumferentially, the penile urethra demonstrated a length of 2.5—3.5 cm (mean 3.1 cm). A urethral defect was created about 1 cm to the external urethral orifice. To guarantee tension-free anastomosis, the urethral defect was bridged with a granulation tissue tube 0.5 cm longer than the excised tissue. In Group 1, a 1-cm urethral defect was repaired with a 1.5-cm granulation tissue tube, with interrupted sutures, using 6/0 Vicryl for anastomosis on both ends. Two non-absorbable sutures using 6/0 Prolene were left on both ends to mark the edges of the graft. In Group 2, a 1.5-cm urethral defect was repaired with 2-cm granulation tissue tube. In Group 3, a 1-cm urethral defect was simply repaired by end-to-end re-anastomosis. For all animals, an 8-Fr urethral catheter was maintained for 14 days postoperatively. Cervical collars were used to prevent animals from removing the urethral catheter. Penicillin G sodium (10^5 u/day intramuscular) was administered for 5 days postoperatively.

Three animals of each group were euthanized at 1, 2 and 6 months postoperatively. At each time point, retrograde urethrograms were firstly performed to assess the urethral caliber. Then the penile urethra was gently dissected from the cavernosa. The urethral lumen was cut longitudinally, and the marking sutures were identified for gross examination and subsequent histological analysis.

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