Reconstruction of the urethral defects with autologous fascial tube graft in a rabbit model

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Abstract

Aim: To investigate the feasibility of the autologous fascia graft in urethra defect reconstruction. Methods: In 24 adult male rabbits, a standardized defect (17 mm) was created within the midportion of each urethra. Two-cm long fascial tube grafts were interposed between the cut ends of the urethra. Twenty-four rabbits were divided into 12 groups. At 0, 3, 10, 15, 21, 30, 45, 60, 90, 120, 150, and 180 days postoperatively, one group was killed. In the first four groups, rabbits were killed and specimens were obtained for histological examination. After 21 postoperative days, in the subsequent eight groups, retrograde urethrograms were carried out to evaluate urethral patency and caliber, then rabbits were killed and specimens were obtained. Results: In the histological study, advancement of the urethral transitional epithelium along scaffold provided by the fascial graft was determined. At the 30th day, the new urethra was completely covered with the transitional epithelium. Fistula formation was observed in two of 24 rabbits. In urethrograms, narrowing was determined in three of 16 rabbits. Conclusion: For segmental urethral reconstruction, fascial graft is a good urethral substitute because of its rapid epithelization capacity, low contraction degree and thinness. We therefore propose the use of fascial grafts for reconstruction of male-urethra defects in humans. (Asian J Androl 2007 Nov; 9: 835–842)

Keywords: urethra; defect; fascia; graft; reconstruction

1 Introduction

Urethral reconstruction is a challenge for reconstructive surgeons. For this aim, skin [1], oral mucosa [2, 3], bladder mucosa [4, 5], digestive mucosa [6], peritoneum [7], vein grafts [8, 9] and preputial tissue flaps [10] have been used. Among these, mucosa and skin grafts and preputial tissue are probably the most popular. While all surgical methods have benefits over the others, they also have problems at different degrees, such as extensive contraction, fistula formation, lack of epithelialization and donor area morbidity. Till now there is still no clear decision on which tissue should be selected for the ure-
Fascia graft for urethral reconstruction.

In this experimental study, we investigated the feasibility of the autologous fascia graft in urethra defect reconstruction because of its rapid epithelization capacity, low degree of contraction and thinness.

2 Materials and methods

2.1 Procedure

Twenty-four New Zealand male rabbits aged between 8–10 months and weighing 3–4 kg were used. Institutional guidelines regarding animal experimentation were followed. The rabbits were housed in an air-conditioned animal facility with 12 h : 12 h light: dark cycles and free access to food and water. Each cage accommodated one rabbit.

Four pilot study procedures were performed prior to the study to perfect operative techniques and plan. A standardized defect (17 mm) was created within the midportion of each urethra. The autologous tensor fascia lata graft obtained from the hindlimbs of the animals was tubularized. Two-cm-long fascial tube grafts were interposed between the cut ends of each urethra.

All rabbits were subjected to the same surgical procedures. General anaesthesia was applied by intraperitoneal injection of 0.5 mL nembutalin under the xiphoid area. The lateral of the thighs and the genital organs of the animals were shaved and cleaned by povidone-iodine solution. The animals were placed on the table in supine position. A 4-cm vertical incision was carried out on the lateral thigh skin and the fascia was reached (Figure 1). A fascia graft measuring $3 \times 2$ cm was harvested. The donor site was closed with 4/0 chrome catgut. The fascial graft was immediately rolled into a tube-shaped around a 6-French gauge urethral catheter using 7/0 vicryl (Figure 2). To move the graft on the catheter easily, the tube graft was purposefully made a little larger in diameter than the catheter.

The urethra was catheterised using a 6-French gauge catheter. A vertical incision was made on the ventral aspect of the penis and ended at 0.5 cm proximal to the distal end. The urethra was separated circumferentially over the catheter between the corporal bodies in the midshaft of the penis. A 17-mm-long midurethral segment was totally excised (Figure 3) and the catheter was taken out. The 2-cm-long fascial tube graft overlying the catheter was then interposed between the cut ends of the urethra (Figure 4). The distal and proximal urethra were reconstructed.
Figure 4. Drawing of the 2-cm-long fascial tube graft overlying the catheter interposed between the cut ends of urethra. The catheter carrying the graft was passed into the proximal and distal ends of the urethra from the defect area.

Figure 5. Before the distal and proximal urethral stumps were anastomosed to the fascia graft.

Figure 6. After the distal and proximal urethral stumps were anastomosed to the fascia graft.

The urethral stumps were anastomosed to the fascia graft with the interrupted sutures by using 7/0 vicryl under the microscope (Figures 5, 6). The subdermal layer was repaired with 7/0 vicryl and the skin was closed with 5/0 chromic catgut. The urethral catheter was stabilised to the distal end of the penis by using 5/0 nylon. The catheter was irrigated twice a day using 1% gentamycin solution. The catheters were removed on postoperative day 7, allowing the animals to urinate spontaneously. Cervical collars were used in the postoperative period to prevent damage to the operation site.

Intravenous hydration (40 mL/h of 5% dextrose in lactated Ringer’s solution) began preoperatively and continued for 2 H to 4 h postoperatively. Antibiotic prophylaxis began preoperatively and continued for 5 days (gentamycin, 5mg/[kg⋅d] intramuscularly).

Twenty-four rabbits were divided into 12 groups. Each group composed of two rabbits. At 0, 3, 10, 15, 21, 30, 45, 60, 90, 120, 150 and 180 days postoperatively, one group was killed. In the first four groups, rabbits were killed then specimens were obtained for histological examination. In the subsequent eight groups, the rabbits first underwent retrograde urethrogram then were killed, and specimens were obtained for histological examination.

2.2 Clinical assessment

Fistula formation and voiding difficulties were examined on the recipient site. Signs of infection and delay in wound healing were investigated on the donor and recipient sites. At the beginning of the histological assessment, the luminal wall was examined to determine the presence of ulceration or polipoid structures.

2.3 Radiological assessment

After postoperative day 21, groups 5–12 (a total of 16 rabbits) underwent retrograde urethrogram under general anesthesia for evaluation of the urethral patency and caliber.

2.4 Histological assessment

At 0, 3, 10, 15, 21, 30, 45, 60, 90, 120, 150 and 180 days postoperatively, the groups 1–12 were killed, respectively, and specimens from 24 rabbits were obtained for histological studies.

In each animal, the penis was removed en bloc, and the operative area was reexposed through the previous incision to obtain the specimen. To evaluate how the
epithelialization proceeds, two specimens were taken longitudinally to the lumen while including the native urethra, anastomosis line and fascial graft. Two cross-sectional specimens were taken from the middle and marginal portions of the graft to incorporate the entire lumen within the slide. The specimens were immediately placed in 10% formalin fixative and then embedded into paraffin. Histological sections were prepared using hematoxylin-eosin staining to evaluate the extent and type of epithelialization.

3 Results

3.1 Clinical assessment

The donor sites exhibited no signs of infection or delay in wound healing. No signs of complete obstruction in the pattern of voiding were detected.

Gross examination made before taking sections for histological assessment showed the absence of ulceration or polipoid structure formation. None of the cases developed hair growth or stone formation. Fascia grafts were intact in all rabbits.

After postoperative day 30, in groups 7–12, the fascia grafts became fully epithelialized and no macroscopical difference was observed between the fascial graft and native urethra. After postoperative day 30, macroscopically, the radius of the lumen was measured as 3–4 mm (min, 2 mm; max, 5 mm) in diameter in 14 rabbits.

Two of the 24 cases developed fistulas. The fistula formation rate was 8%. One of these two animals dislodged the stent before 7 days.

In five cases, the urethral stent was dislodged before 7 days.

3.2 Radiological assessment

Retrograde urethrogram showed normal anatomy in 13 of 16 animals without stricture, papillary hypertrophy or diverticula formation (Figure 7). Different degrees of narrowing were observed in a remaining three of 16 cases. The narrowing rate was 19%. One of these three animals dislodged the stent before 7 days.

Two cases exhibited the narrowing at the proximal anastomosis. One case exhibited the narrowing at the distal anastomosis and fistula formation was associated with the narrowing. The narrowing was medium (approximately 50% reduction at the lumen radius) in one case and slight (approximately 25% reduction at the lumen radius) in two cases (Figure 8).

3.3 Histological assessment

Group 1 (day 0): The urethra and fascia had their own characteristics. These characteristics were used as reference at different periods of healing for the histological evaluation. The urethra had transitional epithelium and fascia had raw surface.

Group 2 (day 3): Acute inflammatory reaction in the wall was detected. Excessive polymorphonuclear leukocyte infiltration was shortly followed by migration of the lymphocytes and macrophages. At the graft surface, fibrinous exudation was present.

Group 3 (day 10): Ingrowing of the urethral epithelium from the margins of the urethra onto the fascia graft was observed, but the epithelium was not multilayered.

Figure 7. Retrograde urethrography obtained at postoperative day 120 demonstrates a satisfactory remodelling of the new urethra with no narrowing.

Figure 8. Retrograde urethrography obtained at postoperative day 60 demonstrates slight (approximately 25% reduction at the lumen radius) narrowing in distal anastomosis line.

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Group 3 (day 10): Longitudinal section including both fascial graft and native urethra. Appearance of ingrowing transitional urethral epithelium from the margins of the urethra onto the fascia graft. Epithelium is not multilayered. Slight fibroblast activation at the subepithelial tissue and leukocyte infiltration in the anastomosis line is observed. Immature collagen fibers are detected (HE, × 40).

(Figure 9). Slight fibroblast activation at the subepithelial tissue and leukocyte infiltration in the anastomosis lines was observed. Immature collagen fibers were detected.

Group 4 (day 15): An epithelial tongue emerging from the surrounding epithelium migrated onto the granulating graft areas. The center of the exposed graft surface was covered with the fibrinous strands. No any other nidus of epithelial cells rather than the wound margins was observed on the granulating graft surface. Surrounding epithelium was hypertrophic. Acute inflammatory reaction still continued. Proliferation of the fibroblasts became dense. Immature collagen fibers became more regular (Figure 10).

Group 5 (day 21): Lumen was almost completely covered with a disorganized multilayered epithelial tissue. The granulation tissue replaced the deep part of fascial graft. Immature collagen fibers in the granulation tissue were arranged in a regular pattern. Decreased acute inflammatory reaction and slight fibroblastic activity in the wall was noted. Neovascularity was visible in the subepithelial layer. Inflammatory reaction persists more prominently at the central region of the graft surface. It was impossible to distinguish the anastomosis lines or the graft itself (Figure 11).

Group 6 (day 30): The lumen was completely covered with highly-organized transitional urethral epithelium. Acute inflammatory reaction was significantly decreased. The granulation tissue had matured to fibrous tissue. A
Fascia graft for urethral reconstruction

regular pattern of collagen fibers in the newly formed fibrous tissue was observed. At the subepithelial area, collagen fibers, slight fibroblastic activity and decreased number of blood vessels was detected (Figure 12).

On postoperative day 10, the urethral epithelium was observed as moved from the anastomosis lines towards the graft. The newly formed epithelium was single layered. After postoperative day 21, the epithelium became multiple layered. After postoperative day 30, the urethra was completely covered with transitional epithelium.

4 Discussion

The urethra is an epithelialized, sterile conduit through which urine flows. Reconstruction of male-urethra defect must restore continuity of the transitional uroepithelium, as this layer prevents stricture formation and infection. In traditional methods of reconstructing the urethra, vascularized local flaps [10], extragenital tissue grafts such as the hairless skin [1], the buccal mucosa [2, 3], and the bladder mucosa [4, 5] are commonly used.

Although skin grafts are commonly used today in urethra reconstruction as they are plentiful and easy to harvest, they have a high complication rate. Skin flaps and grafts employ a cutaneous component that stops uroepithelial migration. Therefore, the main problem of the procedures with the cutaneous component is the absence of the native uroepithelium and risk of stenosis, fistula, hair growth, stone and diverticule formation [11]. Keratinized epithelium of the skin fails to take advantage of the regenerative capability of urethral epithelium. Actually, the urethra has an extensive ability for regeneration. Even when 1/3 or 2/3 of the urethra is removed, the urethra can regenerate itself[12]. However, when removed as a block, fibrous tissue replaces the urethra. Therefore, a substitute that can reproduce the histology of the urethra is necessary to repair the large urethral defect. Because fascia has a high epithelialization capacity [13], we used the bare fascial graft to regenerate the urethral epithelium. As a result, the bare fascia graft provided an environment that allowed the complete regeneration of the urethral epithelium, and the subepithelial area of the fascia graft was replaced by matured fibrous tissue containing regular collagen fibers.

We observed that the healing process of the fascial graft includes the following serial events: (1) inflammatory cell infiltration, (2) granulation tissue formation, (3) epithelial migration, and (4) fibrosis. Inflammation seemed to remodel the extracellular matrices of the grafts by degeneration and fragmentation of their components and resynthesis of new extracellular matrices. This remodelling process is essential for the granulation tissue formation, which is important for epithelial migration. Then an epithelial tongue, originating from the surrounding epithelium, migrated over the granulating grafts and provided epithelialization of fascial grafts. This finding confirmed the hypothesis concerning the origin of the epithelial cells initiating the epithelialization process that bare fascia or muscle flaps act as a scaffold and complete epithelialization from surrounding margins. Epithelial migration occurs because the basal cells lose their attachment to the basement membrane to move onto the wound matrix [14]. After cessation of epithelial migration, the basement membrane and the epithelial differentiation reform [14]. Graft viability is maintained initially by diffusion of nutrients. The ingrowth of vascular tissue was noted at 2 weeks after graft placement.

Another major hypothesis about the neo-uroepithelium formation is the transformation of the graft epithelium into uroepithelium by gradual differentiation [7]. Transformation was observed when using a peritoneal graft as urethral substitute in a rabbit model. The authors reported a gradual differentiation from a single-layer peri-
The bladder mucosa is another graft material. The biology of a bladder mucosal graft studied in a rabbit model revealed that the graft initially undergoes partial degeneration followed by later regeneration. At the postoperative day 8, the mucosal graft varies in thickness, some portions contain only one cell layer. Gradually the mucosa becomes 2–3 cell layers and then resembles a native rabbit urethra with 5–6 cell layers [15]. Despite its resemblance to native urethra, the bladder mucosa has the problem of alteration when it is exposed to air at the tip of the penis. It behaves much like the exposed mucosa of the bladder extrophy and it becomes sticky and hypertrophic. In our study the fascia graft, in contrast to the bladder tissue, showed fast urethral epithelialization without a stage of desquamation or complication, such as thickening of the suture line at the end of the penis, fragility and maceration.

Similar to mucosa grafts, the endothelium of vein grafts undergoes degeneration during the first few days. And when the segmental urethral replacement is in the distal part of the penis there could be some problems up to the third week as a result of exposure of the vein endothelium to air [9]. Because the fascia graft is already raw-surfaced, epithelial degeneration does not occur in fascial graft healing.

Fascia graft harvesting does not require extensive surgery with potential morbidity, such as laparotomy used in the peritoneum, bladder mucosa and colon mucosa harvesting. Tensor fascia lata provides ample graft source with a low donor site morbidity.

In our study, the risk of narrowing and fistula formation in the fascia graft application is 3/16 (19%) and 2/24 (8%), respectively. Two cases exhibiting one fistula and one narrowing formation revealed early dislodgment of the stent. We think that early dislodgment of the stent is a contributing factor for stenosis and fistula formations. The result will be better if the stents can be preserved long enough as the stent provides immediate structural support and ensures direct apposition of the graft to the underlying bed. It is also known that inflammatory reaction persists with prolonged stenting. Seven days is the minimum time for the initial graft uptake and therefore the stent should be kept in place for a minimum of 8 days, and not more than 14 days. Because the fascia graft shows fast epithelialization, 8 days seems adequate for stenting.

Because of the longitudinal suture line, the fistula is a problem for all tube-shaped grafts and flaps. However, the result of the autologous fascia lata patch graft used for the ventral urethral defect in 10 male rabbits exhibited 2/10 (20%) fistula formation [17]. Although this rate (20%) was higher than the fistula formation rate of our study (8%), it cannot be considered high in urethral surgery. The rate of fistula formation and narrowing in clinical studies reporting the use of bladder mucosal grafts has ranged from 0% to 19% [4, 5, 16]. We think that, with further refinements of the techniques, fascia tube grafts can be used safely in humans, with low fistula formation and narrowing rates.

Histologically, results of the autologous fascia lata patch graft studies used for the penile urethral defects in 10 rabbits [17] and 14 dogs [18] were similar to that of our study. Both studies [17, 18] revealed intact lumens covered with transitional epithelium and the graft edges were not detectable.

Kargi et al. [19] showed the successful result of the fascial grafts in the repair of secondary urethral fistulas in eight patients, with no recurrence after 1-year follow-up. They placed 2 × 2-cm fascia lata graft between the
urethra and skin after repair of the fistula.

This present study revealed that fascia tube graft is a good substitute in segmental urethral reconstruction because of its rapid epithelialization capacity with the highly-organized transitional urethral epithelium, thin and pliable nature and low complication rate. In conclusion, this study not only contributes to the urethral reconstruction but also helps us to understand wound-healing mechanisms that will be the basis of tissue-engineering techniques [20].

References


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