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Experimental and clinical experience using tissue regeneration for urethral reconstruction

Abstract Various urethral conditions often require additional tissue for reconstruction. Although several innovative tissues have been proposed for possible use as free grafts for urethral repair, all have specific advantages and disadvantages. The use of these tissues may be associated with additional procedures for graft retrieval, prolonged hospitalization, and donor-site morbidity. For these reasons, alternate materials have been sought for urethral repair. Our laboratory has developed an acellular collagen matrix that has shown adequate urothelial-cell epithelialization and urethral-tissue regeneration both experimentally and clinically. After a 3-year follow-up period, all patients who have had their urethras reconstructed with the acellular matrix are doing well, showing no clinical change from their immediate postoperative results. Other acellular materials may soon be tried clinically. Long-term studies need to be conducted before any of these materials can be accepted for routine use in urethral reconstructive procedures.

Various urethral conditions such as strictures, traumatic defects, congenital defects, and cancer often require additional tissue for reconstruction. In circumstances in which there is a lack of urethral mucosa for adequate reconstruction, tissues from other sources have been used, such as genital and extragenital skin flaps or grafts, mucosal grafts from the bladder or buccal regions, tunica vaginalis, and peritoneal grafts [7, 9, 14].

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Complications such as hair growth, graft shrinkage, stricture, stone formation, and diverticuli have been associated with skin grafts [3, 8, 13, 20, 22]. Bladder-mucosa free grafts have been used for urethral reconstruction; however, problems with mucosal glandular protrusion and donor-site morbidity have limited their applicability [9]. The clinical application of other types of mucosal tissues such as buccal, labial, and rectal grafts has also been tried for urethral repair with various results [7, 10]. The use of a free graft or flap derived from tunica vaginalis has been attempted clinically in hypospadiac patients, but all of these subjects developed meatal stenosis [26].

Although several innovative tissues have been proposed for possible use as free grafts for urethral repair, it is evident that all have specific advantages and disadvantages. The use of these tissues may be associated with additional procedures for graft retrieval, prolonged hospitalization, and donor-site morbidity. For these reasons, alternate materials have been sought for application in urethral repair.

Experimental studies

The use of a variety of synthetic grafts composed of silicone, Teflon, and dacron has been proposed for urethral reconstruction. These materials have been associated with erosion, dislodgement, fistula, stenosis, extravasation, or calcification [1, 11, 12]. Due to the problems associated with the use of nondegradable materials the use of biodegradable substitutes has been pursued. A polyglactin-fiber mesh tube coated with polyhydroxybutyric acid and hyaluronan benzyl ester has been used experimentally. Complete regeneration of the urethral epithelium and the adjacent connective tissue was achieved. The biodegradable polymer meshes served as scaffolds that guided urothelial and connective-tissue regeneration [15, 21].

Nonwoven meshes of polyglycolic acid have been used experimentally [6]. Partial urethrectomies were

performed in rabbits and a segment of the polymer mesh with the appropriate diameter was interposed to form the neourethra in each animal. There was no evidence of voiding difficulty or any other complication. Retrograde urethrograms showed no evidence of stricture formation. Histologic examination of the neourethras demonstrated complete reepithelialization of the polymer-implanted sites by day 14, which continued for the entire duration of the study. Polymer-fiber degradation was evident at 14 days after implantation.

The use of several naturally derived tissue substitutes has been tried in urethral reconstructive procedures. Lyophilized human dura and vein homografts have previously been proposed as guides for transitional epithelium in growth. On implantation in vivo the luminal surface of lyophilized human dura was entirely epithelialized, and the material was absorbed and replaced by granulation tissue [16]. Urethral-tissue replacement using lyophilized vein homografts resulted in the ingrowth of transitional epithelium [18]. Although urothelial-cell epithelialization was observed in these substitute materials, none of them demonstrated urethral smooth-muscle regeneration.

Free grafts of tubularized peritoneum were used experimentally as urethral-tissue substitutes in 12 rabbits. Organized multilayered graft epithelialization occurred; however, fistulas formed in two of the animals [25]. Porcine small-intestinal submucosa (SIS) has recently been used for urethral repair in a rabbit model to determine whether this material might be capable of evoking urethral regeneration. The SIS onlay grafts were shown to promote the regeneration of normal rabbit epithelium supported by a vascularized collagen and smooth-muscle backing. Although the results appear promising, clinical studies have not been reported to date [19].

In our laboratory a naturally derived acellular collagen-based tissue substitute was recently developed from donor porcine bladder (Fig. 1). The acellular collagen matrix had initially been developed in our laboratory as a biomaterial for bladder augmentation. Previous studies performed using this material for bladder augmentation showed that the acellular matrix was biocompatible and was capable of forming bladder tissue similar to the native bladder when implanted in vivo [29]. In a subsequent study we investigated whether the acelluar collagen matrix would be suitable for urethral reconstruction [4].

A ventral urethral defect measuring 1×0.7 cm (approximately one-half of the urethral circumference) was created in ten male rabbits. The acellular collagen matrix was trimmed and used to replace the urethral defect in an onlay fashion. Serial urethrography was performed preoperatively as well as postoperatively at 0.5, 1, 2, 3, and 6 months. Animals were euthanized at 0.5, 1, 2, 3, and 6 months after surgery. The retrieved implants were analyzed grossly, histologically, and immunocytochemically. All animals survived until the time of euthanasia without developing any noticeable voiding dysfunction.

Fig. 1 Acellular collagen matrix

Serial urethrograms confirmed the maintenance of a wide urethral caliber without any signs of stricture. Gross examination at retrieval showed normally appearing tissue without any evidence of fibrosis. At retrieval the distances between the marking sutures placed at the anastomotic margins remained stable, with no distance varying more than 10% in any axis, indicating the maintenance of the initial implant diameter. Histologically, the implanted matrices displayed host-cell infiltration and generous angiogenesis by 2 weeks after surgery. Minimal infiltration of inflammatory cells was initially observed; however, complete disappearance of these cells was evident by the 3-month postoperative time point. There was no evidence of fibrosis or scarring in the urethras at any of the retrieval time points.

The presence of a complete transitional-cell layer over the graft was confirmed at 2 weeks after the repair, and this finding remained consistent throughout the study. The urothelial-cell layers stained positively with the broadly reactive antipancytokeratins AE1/AE3 in all implants. There was no evidence of muscle fibers in implants retrieved at either 2 weeks or 1 month. Unorganized muscle-fiber bundles were evident histologically at 2 months after implantation. The histology patterns suggested that the ingrowth of muscle fibers occurred from all adjacent areas of native tissue, including the ends and sides of the grafts. These findings were confirmed using anti-α-actin antibodies. Increasing numbers of organized muscle bundles were observed at 3 months. Normally appearing organized muscle-fiber bundles were evident at 6 months after implantation (Fig. 2). These results demonstrated that the acellular collagen matrix could be a useful material for urethral repair in the rabbit.

Clinical studies

Cultured autologous cells have been proposed for use in urethral reconstruction [5]. Skin keratinocytes that had

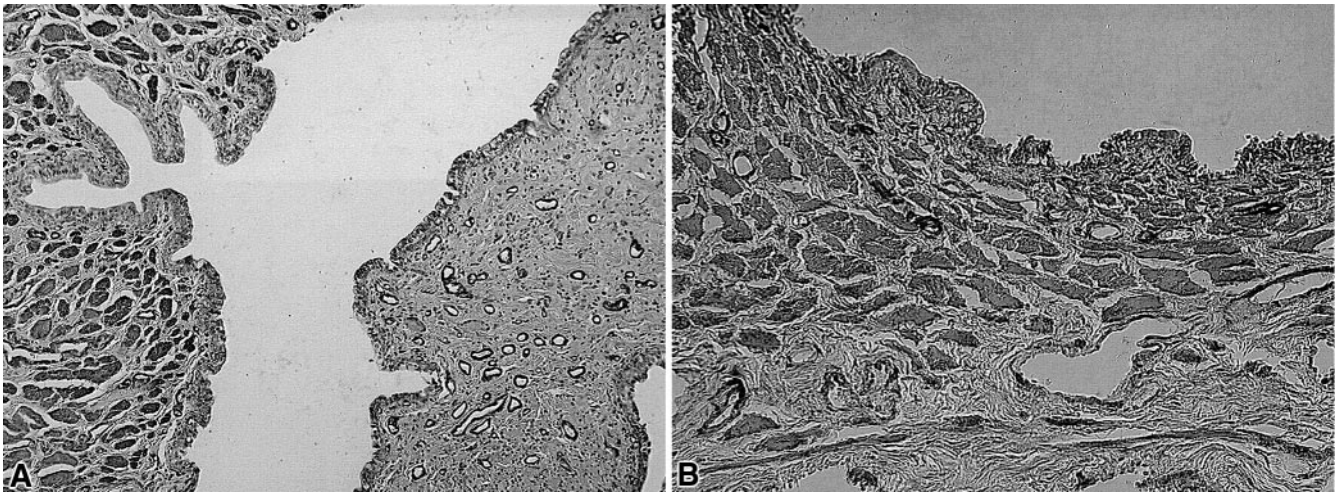

Fig. 2A, B Immunocytochemical analysis with α-actin antibodies. **A** Urethra retrieved at 1 month postsurgery shows a paucity of muscle fibers (*right*). The native portion of the urethra demonstrates the preservation of muscle-fiber bundles (*left*). Reduced from ×40. **B** Normally appearing muscle bundles are visible at 6 months postimplantation. Reduced from ×100

been harvested from the urethral meatus region of two patients were grown in vitro, placed on a graft bed, and used for distal urethral construction [23]. This method resulted in the formation of new urethral tissue; however, a small fistula occurred in both patients. A subsequent study using keratinocytes involved a two-step urethroplasty [24]. An initial urethral meatal biopsy was performed and the keratinocytes were grown in vitro. The expanded cells were seeded on the exterior surface of a tubularized Teflon graft, which was subsequently used for hypospadias repair. A stenosis at the coronal sulcus was observed in all patients.

Tunica vaginalis has been proposed for use as a free graft or a flap for urethral substitution [17, 26–28]. To date, only one report in the literature has described the use of a tunica vaginalis flap in hypospadiac patients, and all of the subjects developed meatal stenosis. Subsequent experimental work using a tunica vaginalis flap in animals demonstrated neourethral contracture [28]. It was suggested that the graft contracture might have been secondary to the presence of striated cremasteric muscle elements associated with the tunica.

After our experimental experience with the collagen-based acellular matrix we used the material clinically for urethral reconstruction [2]. Four patients with a history of hypospadias surgery underwent reoperative repair using the collagen-based matrix for urethral reconstruction. The collagen matrix, which had been obtained from donor cadaver bladder, was processed and trimmed to size as needed for each individual patient. The neourethras were created by anastomosis of the matrix in an onlay fashion to the urethral plate. The size of the resulting neourethra ranged from 5 to 15 cm. After a 22-month follow-up period, three of the four patients had a successful outcome with regard to their cosmetic appearance and function (Fig. 3). One patient who had undergone the creation of a 15-cm neourethra developed a subglanular fistula. These results show that the use of a collagen-based acellular matrix appears to be beneficial for patients with a history of hypospadias repair, who may lack sufficient genital skin for reconstruction. The acellular collagen-based matrix eliminated the necessity of additional surgical procedures for graft harvesting. In addition, the operative time was decreased, as was the potential risk for morbidity due to the harvest procedure.

The acellular matrix was used only in an onlay fashion. The urethral segments reconstructed with the acellular matrices showed a normal cellular organization that was indistinguishable from that of the native urethral tissue. Neither graft contracture nor stricture occurred. It is uncertain whether larger defects could be corrected with the acellular matrix. The maximal area of

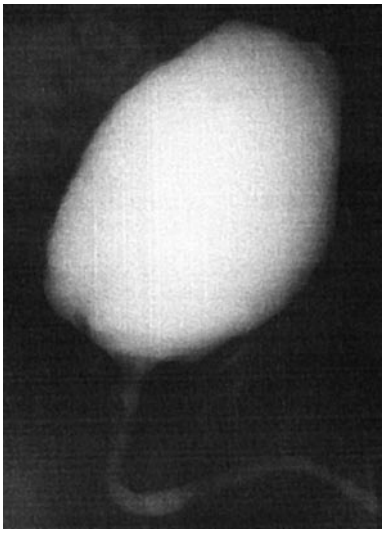

Fig. 3 Postoperative urethrogram obtained in a patient with severe hypospadias following urethral reconstruction with an "off-the-shelf" collagen-based acellular matrix. Maintenance of a normal urethral caliber without any evidence of stricture is apparent at 1 year postsurgery

defect that could be corrected with adequate host-cell infiltration and without graft contracture is unknown. Experiments in our laboratory are currently being conducted to address these questions.

Conclusions

Due to the shortage of urethral tissue available for urethral reconstruction, autologous tissues from various sources have been used. Although each tissue type has specific advantages and disadvantages, it is evident that these tissues are associated with additional procedures for graft retrieval, prolonged hospitalization, and donor-site morbidity. The ready availability of an "off-the-shelf" material for urethral reconstruction would eliminate these problems and provide an attractive option. Toward this end, several urethral-tissue substitute materials have been proposed over the years.

Our laboratory has developed an acellular collagen matrix that has shown adequate urothelial-cell epithelialization and urethral-tissue regeneration both experimentally and clinically. After a 3-year follow-up period, all patients who had their urethras reconstructed with the acellular matrix are doing well, showing no clinical change from their immediate postoperative results. The acellular collagen matrix may have several advantages over the tissues currently used for urethral reconstruction. The matrix can be processed easily, has good characteristics for tissue handling and urethral function, and has the advantage of being an "off-the-shelf" material. The use of other acellular materials may soon be tried clinically. Long-term studies need to be conducted before any of these materials can be accepted for routine use in urethral reconstructive procedures.

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