Novel Concept and Method of Endoscopic Urethral Stricture Treatment Using Liquid Buccal Mucosal Graft

Dmitriy Nikolavsky,* Jared Manwaring, Gennady Bratslavsky, Tiffany Caza, Steve Landas, Anita Hryniewicz-Jankowska and Leszek Kotula

From the Departments of Urology and Pathology (TC, SL), State University of New York Upstate Medical University, Syracuse, New York

Abbreviations and Acronyms

BMG = buccal mucosa graft

DVIU = direct vision internal urethrotomy

RUG = retrograde urethrogram

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* Correspondence: Department of Urology, State University of New York Upstate Medical University, 750 East Adams St., Syracuse, New York 13210 (telephone: 248-837-0404; e-mail: nikolavd@upstate.edu).

Purpose: We describe a novel method of urethral stricture treatment using liquid buccal mucosal grafts to augment direct vision internal urethrotomy.

Materials and Methods: A rabbit stricture model was used to test this method. In phase 1 the concept of endoscopic liquid buccal mucosal graft implantation was tested by performing direct vision internal urethrotomy in 3 rabbits with immediate intraurethral injection of autologous liquid buccal mucosal grafts suspended in fibrin glue. Animals were sacrificed at 2 to 3 weeks and the urethras were examined for the presence of buccal mucosa engraftment. In phase 2 strictures were induced by electroresection in 9 rabbits divided into 2 groups, including 1) 6 rabbits treated with direct vision internal urethrotomy and liquid buccal mucosal grafts, and 2) 3 controls that underwent direct vision internal urethrotomy and injection of fibrin glue only. Two treated and 1 control animals were sacrificed at 8, 16 and 24 weeks each. Prior to sacrifice the animals underwent retrograde urethrograms and urethroscopy. Histological specimens were examined for the presence of buccal mucosal engraftment.

Results: In phase 1, 2 of the 3 rabbits demonstrated engraftment of buccal mucosa in the urethra after injection of liquid buccal mucosal grafts. In phase 2 all 6 treated animals demonstrated engraftment with resolution/improvement of strictures on retrograde urethrograms and urethroscopy. Controls had no buccal engraftment and showed fibrosis and chronic inflammation. One of the 3 controls had persistent stricture on retrograde urethrograms and cystoscopy.

Conclusions: This proof of concept study demonstrated the feasibility of using liquid buccal mucosal grafts for endoscopic urethral stricture repair. Such a method may allow for wide application of this novel concept of using liquid buccal mucosal grafts to augment direct vision internal urethrotomy.

Key Words: urethral stricture, grafts, mouth mucosa, injections, minimally invasive surgical procedures

The 2 most debated treatments for urethral stricture disease are commonly performed endoscopic DVIU or more effective but less commonly performed open urethral reconstruction, that is urethroplasty. Conceptually, the similarity between DVIU and open augmentation urethroplasty (ie BMG onlays or inlays) is urethrotomy, which is done to widen the lumen of the urethra. The key difference between these competing techniques is the presence or the absence of the BMG covering in the created spongiosal defect.

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While DVIU is an endoscopic procedure, it has a high failure rate.[1,2] Open urethroplasty is more successful but it is typically considered a more invasive approach. Prior attempts at endoscopic delivery and fixation of the grafts to cover the defect did not gain wide acceptance due to significant technical difficulties.[3]

The aim of this experimental study was to combine the key parts of the 2 competing procedures and produce a technically simple, minimally invasive endoscopic urethroplasty using augmentation with liquid BMG. We hypothesized that autologous buccal mucosal fragments suspended in liquid (liquid micrografts) could be implanted in the endoscopically created defect in the urethra and be used to treat urethral stricture as a BMG augmented DVIU.

MATERIALS AND METHODS

A rabbit animal model was used in our experimental design. All experiments were done after review and approval by the institutional animal care and use committee in accordance with the Animal Welfare Act.

Phase 1

Phase 1 consisted of liquid graft implantation in a defect in a healthy urethra as proof of principle.

Urethral Defect Creation. Four postpubertal male New Zealand white rabbits (Charles River Laboratories International, Wilmington, Massachusetts) weighing 2.8 to 3.4 kg were used. After the standard 1-week acclimatization period anesthesia was induced with 40 mg/kg ketamine hydrochloride and 6 mg/kg xylazine intramuscularly. The genitalia were prepared with povidone-iodine solution. A 10Fr pediatric urethrotome (Karl Storz, Berlin, Germany) was used to create a 1 cm longitudinal urethral mucosal incision ventrally at the 6 o'clock position into the corpus spongiosum. The location of the defect was 2 to 3 cm proximal to the meatus and 1 cm distal to the membranous urethra. A 10Fr silicone catheter was then placed in the bladder and the balloon was inflated with 3 cc sterile water.

Liquid Buccal Mucosal Micrograft Preparation. A BMG 8 mm in diameter (50.3 mm^2) was harvested using a circular punch biopsy (Miltex, York, Pennsylvania). The mucosa was rinsed in antibiotic solution containing 100 mg/l gentamicin and 1 gm/l vancomycin. The mucosa was defatted and mechanically minced in 1 cc antibiotic solution to create less than 1 mm fragments with sterile No. 15 surgical blades in a sterile metal dish (fig. 1). The minced mucosa was rinsed in a centrifuge tube and spun for 5 minutes at 3,400 rpm to form a pellet. A carrier liquid for pellet resuspension was prepared by diluting 1:1 the individual components of the surgical fibrin glue Tisseel® with the antibiotic solution. The tissue was then resuspended in 2 separate aliquots in 1 cc Eppendorf tubes, including 1 tube containing 150 μl fibrinogen and the other containing 150 μl thrombin (Tisseel components).

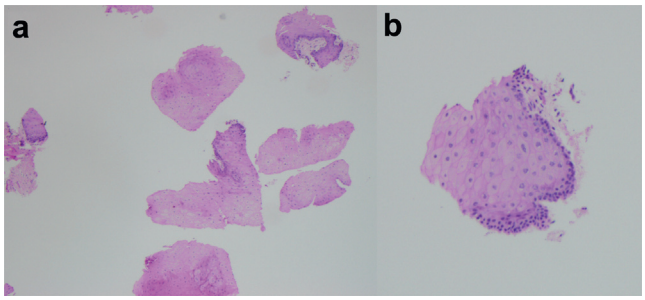

Figure 1. Minced sub mm buccal mucosa micrografts. *a*, H&E, reduced from ×50. *b*, higher magnification shows fragment with smaller basal cells thought to be progenitor cells and larger differentiated cells. H&E, reduced from ×100.

Liquid Buccal Micrograft Application. To prevent solidification of the carrier glue in the syringe each aliquot was injected separately in the urethra. Thrombin-BMG solution was injected first.

A 14 gauge angiocatheter fitted on a 1 cc syringe was placed through the meatus adjacent to the Foley catheter and the suspended micrograft solutions were injected. The Foley catheter was then secured in place with nonabsorbable monofilament suture and further protected with elastic wraps on the abdomen. Animals were fitted with neck cones until the catheters were discontinued 1 week postoperatively.

Specimen Collection. The animals were sacrificed 3 weeks postoperatively by a lethal injection of 390 mg pentobarbital per 10 pounds. Whole urethras and penises were harvested en bloc immediately after sacrifice. In detail, penoscrotal skin webs were incised to release the penis. The penis was degloved and circumferentially dissected to the level of the pubic ramus. The entire specimen was transected at the level of the membranous urethra, suspended in 10% neutral buffered formalin and sent for histological examination by a urological pathologist.

Phase 2

Phase 2 consisted of the buccal mucosal liquid micrograft for the treatment of urethral stricture.

Stricture Induction. A total of 12 male New Zealand white rabbits (Charles River Laboratories International) weighing 2.8 to 3.6 kg were acclimatized for 1 week per protocol. Urethral strictures were induced in these rabbits according to a modification of the electroresection protocol described by Faydaci et al.[4]

Specifically, anesthesia was induced as described. The abdomen was shaved to place a pediatric grounding pad (ConMed, Utica, New York). Anesthesia was maintained with inhaled isoflurane. Prior to resection RUG was performed under fluoroscopic guidance using 240 mgI/ml iohexol nonionic radiographic contrast material (Omnipaque™) to establish a baseline.