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Development of a porcine animal model for urethral stricture repair using autologous urothelial cells

J. Seibold, C. Selent, G. Feil, J. Wiedemann, D. Colleselli, J. Mundhenk, G. Gakis, K.-D. Sievert, C. Schwentner*, A. Stenzl

Department of Urology, Eberhard-Karls University, Tuebingen, Germany

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Abstract *Objective:* To present a versatile large animal model for endoscopic stricture repair using autologous urothelial cells.
Materials and methods: 12 male minipigs were used. An artificial stricture model was established using suture-ligation, thermo-coagulation and internal urethrotomy. A vesicostomy served for urinary diversion. Stricture formation was confirmed radiologically and histologically. Autologous urothelial cells were harvested from bladder washings, cultivated and labeled. Internal urethrotomy was done in all, and the cultivated cells were injected into the urethrotomy wound. All animals were sacrificed after 4 or 8 weeks. Immunohistology was done to confirm the presence of autologous urothelial cells within the reconstituted urethra.
Results: Stricture formation was verified with all three methods. Histologically, no significant differences in the severity of stricture development could be observed with regard to the method used. The autologous urothelial cells in the area of the urethrotomy could be detected in the urothelium and the corpus spongiosum until 8 weeks after re-implantation.
Conclusions: We created a reliable and reproducible porcine model for artificial urethral strictures. Autologous urothelial cells can be implanted into an artificial stricture after urethrotomy. These cells retain their epithelial phenotype and are integrated in the resident urothelium. Further comparative studies are needed to ultimately determine a superior efficacy of this novel approach.

* Corresponding author. Department of Urology, University Hospital Tuebingen, Hoppe-Seyler Strasse 3, 72076 Tuebingen, Germany.
E-mail address: christian.schwentner@med.uni-tuebingen.de (C. Schwentner).

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Introduction

From a histological point of view, a urethral stricture is marked scarring leading to a reduction of the urethral lumen. Alteration of the urothelium is accompanied by a modification to the matrix composition of the subepithelial connective tissue. In particular, the shift in the collagen ratio (mainly type I and III) results in reduced elasticity of the urethra [1]. This alteration also involves the corpus spongiosum, being then referred to as spongiofibrosis. Diminished urinary stream, pain at voiding and increased susceptibility to infections are characteristic.

The actual etiology of urethral stricture development cannot be clarified completely in many cases. In the course of the last decades, a shift in the etiology of urethral strictures is evident. The data show a reduction in strictures caused by infection whereas the number of iatrogenic strictures due to transurethral surgical interventions, e. g. during insertion of a urethral catheter or endoscopic surgery, has markedly increased [2].

In the clinical routine, direct vision internal urethrotomy is usually performed first although the recurrence rate is approx. 90% in repeat cases. In longer or recurrent strictures, open excision of the stricture with subsequent reanastomosis (end-to-end anastomosis) is favored. In larger defects, coverage by means of autologous transplants, e.g. buccal mucosa, is chosen for the surgical procedure [3]. Disadvantages of the utilization of buccal mucosa are scar formation in the area of the submucosa, pain, numbness at the graft donor site, reduced mandible movements, as well as injuries to the salivary glands [4,5]. In more complex cases, the two-stage operative technique (according to Bracka) or a combination of two transplants (inner prepuce and buccal mucosa) is employed [2]. Often, autologous transplants are the only therapeutic option for long (recurrent) strictures and their success can only be fully evaluated after a period of at least 5 years [6]. Considering the poor long-term results of internal urethrotomy and the relative morbidity of open graft urethroplasty, the therapeutic optimization of the open, and very laborious, treatment of strictures remains desirable. For the development of alternative techniques reliable animal stricture models are required. Synthetically produced materials are not suitable for urethral replacement because they result in incrustation, transplant shrinkage, recurrent strictures and formation of calculi [7]. During the last 10 years, new treatment concepts on the basis of tissue engineering have been developed for the therapy of urethral strictures. Different strategies with homologous and heterologous acellular matrices have been investigated in animal models as well as in humans [8–12]. It can be observed that unpopulated matrices are less suitable for the therapy of strictures compared to populated matrix constructs. This could be attributed to poor inosculation and formation of recurrent strictures. However, even composite acellular matrix compositions can involve complications such as fibrosis and shrinkage [11]. These data show that an optimal method for the treatment of urethral strictures has not been found yet. Established procedures for the therapy of urethral defects by means of autologous urothelial cells do not currently exist.

The aim of this study was to establish a large animal model as a preliminary stage for the clinical implementation of in-vitro cultured autologous urothelial cells for the treatment of urethral stricture disease. Moreover, the feasibility of injection of such cultured cells and an appropriate carrier into a urethrotomy wound should be evaluated. For that purpose, adhesion of the urothelial cells in the area of the incised scar was examined to analyze whether the transplanted cells survived and integrated into the scar.

Methods

The study comprised 12 male Göttingen minipigs (Ellegaard, Dalmose, Denmark) at the age of 9 months with a body weight of 19–21 kg. The animals were treated according to local animal healthcare committee approval (CU 1/05). First, the ability of three different methods (ligation, thermo-coagulation and internal urethrotomy) to reliably establish urethral strictures in a large animal model was investigated.

Artificial stricture placement

Urethral strictures were placed in the distal urethra by: ligation with a non-absorbable polypropylene suture (Prolene®, Ethicon, Johnson & Johnson MEDICAL GmbH, Norderstedt, Germany); thermo-coagulation using a hook-shaped ball end Force 2 electrode (Valleylab Inc, Boulder, CO, USA) at 80 W; or by internal urethrotomy using a 9 F pediatric cystoscope (Karl Storz, Tuttlingen, Germany) (Fig. 1). Cutaneous vesicostomy was used for permanent urinary diversion. Stenosis was controlled by urethrography. For this purpose, 10 ml contrast media (Peritrast Infusio 31% (retro), Dr. F. Köhler Chemie GmbH, Bensheim, Germany) were instilled in the urethra and the urinary bladder. For imaging, the system BV Pulsera (Philips Medical Systems, Eindhoven, Netherlands) was used. The animals were sacrificed 1, 8 and 12 weeks later. The urethra was removed for histological evaluation of stricture formation and potential inflammation. Cryostat sections (5 μm in thickness) were stained with hematoxylin and eosin (Vector Laboratories Inc., CA, USA). To examine the extent of fibrosis, Heidenhain's azan stain was applied (Fig. 2).

Isolation and culture of porcine urothelial cells

Bladder specimens obtained from vesicostomy were prepared as described previously for human urothelial cell culture [23,24] (Fig. 1). Briefly, specimens of ~2 cm^2 were transferred into a stripping solution of Hank's balanced salt solution with 0.35 g/l $NaHCO_3$ and phenol red, and without Ca^{2+} and Mg^{2+} (Biochrom, Berlin, Germany) containing 10 mmol/l 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Gibco, Karlsruhe, Germany), 20 KIU/ml aprotinin (Calbiochem, Merck, Darmstadt, Germany), and 1% ethylene-diamine-tetraacetic acid (EDTA) without Ca^{2+} and Mg^{2+} (Biochrom). After incubation for 2 h at 37 °C and 5% CO_2 the specimens were transferred into complete keratinocyte serum-free medium (cKSFM) with bovine pituitary