

RECONSTRUCTIVE UROLOGY

Tissue engineering of urethra using human vascular endothelial growth factor gene-modified bladder urothelial cells

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Acquired or congenital abnormalities may lead to urethral damage or loss, often requiring surgical reconstruction. Urethrocutaneous fistula and strictures are common complications, due to inadequate blood supply. Thus, adequate blood supply is a key factor for successful urethral tissue reconstruction. In this study, urethral grafts were prepared by seeding rabbit bladder urothelial cells (UCs) modified with human vascular endothelial growth factor (VEGF(165)) gene in the decellularized artery matrix. A retroviral pMSCV-VEGF(165)-GFP vector was cloned by insertion of VEGF open reading frame into the vector pMSCV-GFP (murine stem cell virus [MSCV]; green fluorescent protein [GFP]). Retrovirus was generated using package cell line 293T. Rabbit UCs were expanded *ex vivo* and modified with either MSCV-VEGF(165)-GFP or control MSCV-GFP retrovirus. Transduction efficiency was analyzed by fluorescence-activated cell sorting. The expression of VEGF(165) was examined by immunofluorescence, reverse transcript-polymerase chain reaction, Western blot, and enzyme-linked immunosorbent assay (ELISA). Decellularized rabbit artery matrix was seeded with genetically modified UCs and was subsequently cultured for 1 week prior to subcutaneous implantation into nude mice. Four weeks after implantation, the implants were harvested and analyzed by fluorescence microscopy, and by histologic and immunohistochemical staining. *Ex vivo* transduction efficiency of UCs was greater than 50% when concentrated retrovirus was used. The modified cells expressed both VEGF and GFP protein. Furthermore, the VEGF-modified UCs secreted VEGF in a time-dependent manner. Scanning electron microscopy and histochemical analysis of cross sections of the cultured urethral grafts showed that the seeded cells were attached and proliferated on the luminal surface of the decellularized artery matrix. In the subcutaneously implanted vessels, VEGF-modified cells significantly enhanced neovascularization and the formation of a urethral layer compared to GFP-modified cells. These results indicate that VEGF gene therapy may be a suitable approach to increase the blood supply in tissue engineering for treatment of urethral damage or loss.

Editorial Comment

The regeneration of urethral strictures remains a challenge with different approaches being taken to improve the long-term outcome. In most cases buccal mucosa is the current gold standard (1). However, other

approaches continue to be investigated so that a second surgical location can be avoided which would significantly decrease the patient's discomfort and other potential postoperative risks.

In recent years, these approaches have focused on efforts to simplify the surgical approach through the research of shelf-prepared material. In its initial stages, we began to use an organ specific acellular matrix and found during regeneration that certain growth factors change significantly over time (2). More recently, different approaches have been taken in order to overcome the well-known problem of back-drafts that occur during the regeneration process (3).

The authors have advanced the regeneration process with the use of a seeded acellular artery matrix using VEGF-expressing urothelial cells to improve the outcome for sustained urothelial reconstruction; the results have been positive and resulted in a faster angiogenesis of the acellular matrix so that an almost normal urethra has been created, which has been previously investigated for bladder regeneration (4). Basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), platelet derived growth factor-BB (PDGF-BB), vascular endothelial growth factor (VEGF), insulin like growth factor-1 (IGF-1) and heparin binding epidermal growth factor (HB-EGF) are involved during angiogenesis and inhibited significantly graft shrinkage (5).

However, despite the fact that certain growth factors are a necessity, today we (as the authors critically self comment) still do not know which cells are influencing the surrounding tissue. The reported approach could be beneficial, if it is possible that the acting growth factors can be timed so that possible side effects are limited similar to the demonstrated turnover of tissue engineered urothelium cells (6). Conversely, we have been able to culture and stratify a multi-layer urothelium out of urothelium cells harvested from a bladder wash that might further improve regeneration (7). With these two different strategies, we must always bear in mind that the acellular matrix used should be as similar as possible to support the best regeneration that also further demonstrates its influence in the re-vascularization process (8).

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Early continence outcomes of posterior musculofascial plate reconstruction during robotic and laparoscopic prostatectomy

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Objectives: To detail the technique and evaluate in a preliminary study the effectiveness of posterior reconstruction of Denonvilliers' musculofascial plate (PRDMP) in enhancing early continence after robotic and laparoscopic radical prostatectomy (RP).

Patients and Methods: Thirty-two consecutive patients having robotic or laparoscopic RP with PRDMP (group 1). Thirty previous patients not having PRDMP were compared as historical controls (group 2). Continence, as measured by patient self-reporting of the number of pads used/24 h, was assessed at 3 days and 6 weeks after catheter removal, by telephone interview. 'Continent' was defined as the use of none or one pads, 'moderate incontinence' as two pads, and 'severe incontinence' as more than two pads. Intraoperative transrectal ultrasonography (TRUS) was used to measure the membranous urethral length before and after PRDMP.

Results: At 3 days after catheter removal, more patients in group 1 were continent than in group 2 (34% vs 3%, $P = 0.007$). At 6 weeks continence was again better in group 1 (56% vs 17%, $P = 0.006$). The mean length of the membranous urethra on TRUS measured before RP, after RP but before the musculofascial suture, and afterward, was 15.6, 12 and 14 mm, respectively. Thus, reconstruction restored the length of the transected membranous urethra by a mean of 2 mm.

Conclusions: PRDMP during robotic and laparoscopic RP leads to improved maintenance of membranous urethral length and significantly higher early continence rates.

Editorial Comment

At the end of the 21st century, the first reports about the external urethral sphincter or rhabdosphincter can be found which discuss omega-shaped of striated muscle fibers innervated by the pudendal nerves (1,2). With further investigations it was demonstrated that the preservation of the levator ani fascia helps to protect the levator ani muscle, rhabdosphincter with its pudendal nerve branches (3). With the reconstruction of the posterior fibrous raphe, early continence was revealed (4). The propagation of these findings for the robotic or laparoscopic radical prostatectomy (RP) results in similar findings as previously reported for the open RP (5).

Similar results seem to be achieved with the reported preservation of the periprostatic nerve courses as recently described by Hennenlotter et al. 2007, which might further avoid the discontinuation of the posterior fibrous raphe (6). Therefore it might be suggested that the careful preservation of the extracapsular nerve courses especially at the apex might be supportive for the sphincter function especially in the early postoperative phase.

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