RECONSTRUCTIVE UROLGY

Biodegradable urethral stents seeded with autologous urethral epithelial cells in the treatment of post-traumatic urethral stricture: a feasibility study in a rabbit model

Fu WJ, Zhang X, Zhang BH, Zhang P, Hong BF, Gao JP, Meng B, Kun H, Cui FZ
Department of Urology, Chinese People's Liberation Army General Hospital, Military Postgraduate Medical College, Beijing, People's Republic of China
BJU Int. 2009; 104: 263-8

Objective: To evaluate the adhesion and growth of rabbit urethral epithelial cells (UECs) on a biodegradable unbraided mesh urethral stent, and to assess the feasibility and effect of the cell-seeded urethral stent for treating post-traumatic urethral stricture (PTUS) in a rabbit model.
Materials and Methods: Rabbit UECs were collected by biopsy from adult rabbit urethra and seeded onto the outer layer of a mesh biodegradable urethral stent. The growth of UECs in cell-scaffolds was assessed by scanning electron microscopy, immunohistochemical and fluorescence staining. In all, 32 male New Zealand rabbits were used, with either PTUS or uninjured, as a control group. Cell-seeded stents were implanted into the rabbits strictured urethra. The histological and immunohistochemical findings were assessed after death at 1, 2, 8, 12 and 24 weeks, respectively. The reconstruction and function were evaluated by urethroscopy and retrograde urethrography.
Results: The cultured UECs adhered to the stent and grew well. Immunohistochemistry showed that the cells were stained positively for cytokeratin. At 4 weeks, vs. 2 weeks, the thickness of the papillary projections of the epithelium decreased and inflammatory cell infiltration diminished. At 24 weeks the injured urethra was completely covered by integrated regeneration of three to five layers of urothelium. There was no evidence of voiding difficulty, stricture recurrence or other complications.
Conclusions: The unbraided mesh biodegradable urethral stent with autologous UECs seemed to be feasible for treating PTUS in the rabbit urethra, and provides a hopeful avenue for clinical application allowing reconstruction of PTUS.

Urethral replacement using cell seeded tubularized collagen matrices

De Filippo RE, Yoo JJ, Atala A
Department of Urology, Children's Hospital and Harvard Medical School, Boston, MA, USA
J Urol. 2002; 168:1789-92; discussion 1792-3

Purpose: Acellular collagen matrices derived from bladder submucosa have been used successfully as an off-the-shelf biomaterial for urethral replacement, experimentally and clinically in an onlay fashion. We investigated whether collagen matrices, either alone or with autologous cells, could be used for tubularized urethral replacement.
Materials and Methods: Acellular collagen matrices were processed and tubularized. Ten rabbits underwent an open bladder biopsy with subsequent cell expansion. Autologous bladder cells were grown and seeded onto the pre-configured tubular matrices. A 1 cm. long urethral segment was excised in 24 male rabbits. Urethroplasty was performed with the tubularized collagen matrices seeded with cells in 12 animals and without cells in 12. Serial urethrography was performed preoperatively and at 1, 2, 3 and 6 months postoperatively. Retrieved urethras were analyzed grossly, histologically, immunocytochemically and with Western blots. Contractility and the presence of neurotransmitter receptors were confirmed with organ bath studies.
Results: Serial urethrography confirmed the maintenance of a wide urethral caliber without any signs of strictures in animals implanted with the cell seeded matrices. The urethral segments replaced with the collagen

scaffolds without cells demonstrated strictures and graft collapse at all time points. The implanted cell seeded matrices had a normal urethral architecture by 1 month, consisting of a transitional cell layer surrounded by muscle cell fiber bundles with increasing cellular organization with time. Epithelial and smooth muscle phenotypes were confirmed immunocytochemically and with Western blot analyses using pancytokeratins AE1/AE3 and smooth muscle specific alpha-actin antibodies. Formation of a transitional cell layer was confirmed in the matrices implanted without cells but only scant unorganized muscle fiber bundles were present, mostly at the anastomotic sites. Organ bath studies demonstrated the capacity for contractility along with cholinergic and adrenergic specific receptors in the tissue engineered scaffolds compared to controls.
Conclusions: These results show that collagen matrices seeded with cells form normal urethral tissue can be used for tubularized replacement, whereas tubularized collagen matrices alone without cells lead to poor tissue formation and strictures. The collagen matrices seeded with cells may offer a useful alternative in the future for patients requiring a tubularized urethral segment replacement.

Tubularized urethral replacement with unseeded matrices: what is the maximum distance for normal tissue regeneration?

Dorin RP, Pohl HG, De Filippo RE, Yoo JJ, Atala A
Department of Urology, LAC+USC Medical Center, Los Angeles, CA, USA
World J Urol. 2008; 26: 323-6

Purpose: Complete urethral replacement using unseeded matrices has been proposed as a possible therapy in cases of congenital or acquired anomalies producing significant defects. Tissue regeneration involves fibrin deposition, re-epithelialization, and remodeling that are limited by the size of the defect. Scar formation occurs because of an inability of native cells to regenerate over the defect before fibrosis takes place. We investigated the maximum potential distance of normal native tissue regeneration over a range of distances using acellular matrices for tubular grafts as an experimental model.
Materials and Methods: Tubularized urethroplasties were performed in 12 male rabbits using acellular matrices of bladder submucosa at varying lengths (0.5, 1, 2, and 3 cm). Serial urethrography was performed at 1, 3, and 4 weeks. Animals were sacrificed at 1, 3, and 4 weeks and the grafts harvested. Urothelial and smooth muscle cell regeneration was documented histologically with HE and Masson's trichrome stains.
Results: Urethrograms demonstrated normal urethral calibers in the 0.5 cm group at all time points. The evolution of a stricture was demonstrated in the 1, 2, and 3 cm grafts by 4 weeks. Histologically all grafts demonstrated ingrowth of urothelial cells from the anastomotic sites at 1 week. By 4 weeks, the 0.5 cm grafts had a normal transitional layer of epithelium surrounded by a layer of muscle within the wall of the urethral lumen. The 1, 2, and 3 cm grafts showed ingrowth and normal cellular regeneration only at the anastomotic edges with increased collagen deposition and fibrosis toward the center by 2 weeks, and dense fibrin deposition throughout the grafts by 4 weeks.
Conclusions: The maximum defect distance suitable for normal tissue formation using acellular grafts that rely on the native cells for tissue regeneration appears to be 0.5 cm. The indications for the use of acellular matrices in tubularized grafts may therefore be limited by the size of the defect to be repaired.

Editorial Comment

When no autologous tissue is available for reconstruction of the urethra from hypospadias or urethral stricture disease, tissue engineering provides an alternative. These 3 articles summarize the current state of tissue engineering in the urethra.

There are two basic components to tissue engineering: the acellular matrix and the cellular epithelium. To avoid rejection it is important that the cells populating the engineered tissue are the patient's own (autolo-

gous). In the case of urethral replacement, these are commonly derived from culturing transitional epithelial cells obtained from a bladder biopsy. Still, these cells cannot simply be injected into the diseased urethra with any hope of successful implantation and generation of a normal appearing urethra. Instead, their growth and differentiation must be supported by a tissue matrix. The extracellular matrix comes in two varieties: an acellular heterologous collagen matrix or a biodegradable synthetic polymer matrix. Examples of collagen matrices include small intestinal submucosa (SIS) and bladder collagen matrix. The synthetic matrices are composed of polymers such as polylactic acid that can be degraded by enzymatic hydrolysis into non-toxic byproducts: carbon dioxide and water. The purpose of the extracellular matrix (whether collagen or polymer) is to provide mechanical and architectural support for native cellular ingrowth. These matrices are biodegradable so that as the patient generates his new urethra, the foreign material is resorbed.

These 3 articles tell the story of the principles that have been discovered to govern urethral engineering thus far. First, acellular matrices have been successfully used in an onlay fashion by themselves (without seeding them with transitional cells). It appears that as long as there is normal urethral epithelium along the edges of the onlay matrix graft, these cells can grow in from the edges and populate the graft. However, when matrix grafts have been used as a tubular graft (i.e. complete urethral replacement) only very short graft have been successful (0.5 cm in animal models). The utility of such short tube grafts is questionable as short defects can be bridged generally with primary anastomosis of the native urethra. Tubular grafts will likely serve their role in complete replacement of longer segments of severe urethral disease when onlay options are not available or feasible due to a lack of a dorsal plate. In such cases, it is clear that whether a biodegradable synthetic mesh or an acellular heterologous matrix graft is used it will be necessary to seed these grafts with epithelial cells.

Continued investigation in animal models and human trials will expand the role of tissue engineering for salvaging the devastated urethra.

Dr. Sean P. Elliott
Department of Urology Surgery
University of Minnesota
Minneapolis, Minnesota, USA
E-mail: selliott@umn.edu

UROLOGICAL ONCOLOGY

High frequency of intracerebral hemorrhage in metastatic renal carcinoma patients with brain metastases treated with tyrosine kinase inhibitors targeting the vascular endothelial growth factor receptor

Pouessel D, Culine S
Department of Medical Oncology, C.R.L.C. Val d'Aurelle, Montpellier, France
Eur Urol. 2008; 53: 376-81

Objectives: To report the high incidence of intracerebral hemorrhage (ICH) in patients with metastatic renal cell carcinoma (RCC) treated with the tyrosine kinase inhibitors targeting the vascular endothelial growth factor receptor (VEGFR).
Methods and Results: Between October 2005 and December 2006, 67 patients with metastatic RCC were treated with sorafenib or sunitinib at the Montpellier Cancer Center in compassionate access programs. The