

Chapter 7

Ureter

Tissue-engineered ureter using a decellularized matrix

Partial ureterectomy is performed for patients with ureteral cancer or severe retroperitoneal fibrosis [1, 2]. When the length of the dissected ureter is long, especially proximally, ureteral replacement is required to avoid urinary diversion, such as nephrostomy or autorenal transplantation. However, previous trials using artificial materials have failed due to infection, inflammation, or calcification [3-7]. To improve the quality of life for patients undergoing nephrostomy, development of a novel graft material is needed. Decellularized matrices have been widely used as scaffolds for tissue engineering. Compared with biodegradable polymers, decellularized matrices possess physiological properties that are closer to those of normal tissue. These properties are beneficial for forming tubular structures, such as blood vessels, with similar pliancy to that of normal vessels [8]. Furthermore, decellularized matrices are highly biocompatible, which facilitates reepithelialization after transplantation [9]. This is of great benefit when a scaffold requires an epithelial lining. Decellularized matrices are also known to be minimally immunogenic, even in the case of xenografts, which is advantageous when a stable supply of scaffold material is required for clinical applications [10, 11]. One of the most important factors for successful tissue-engineered ureters is thought to be a functional lining with uroepithelial cells (UECs), which are expected to maintain the luminal space, prevent calculus formation, and possibly resist stricture of the duct [3-6]. Fortunately, UECs from the bladder are readily available clinically, by endoscopy, without radical surgical procedures. Thus, the combination of an allogenic or xenogenic decellularized ureter with autologous UECs might generate an ideal material for grafting. Decellularized matrices have been used to construct tissue-engineered urinary bladders and ureters in rat models [9-12]. However, replacement of the ureter in larger animals has not yet been successful [13]. We suggest that one of the major obstacles to application with the large animal models is providing a blood supply for the grafted epithelial cells, as the thickness of the scaffold in animals such as dogs is above the limit of diffusion for nutrients and oxygen from the surrounding tissue, causing the grafted cells to starve. Unlike tissue-engineered blood vessels, tissue-engineered ureters cannot access a blood supply until neovascularization is completed. In fact, our preliminary experiments have shown that epithelial cells seeded onto canine ureteral decellularized matrices (UDMs) gradually disappear over the 14-day period after transplantation. Several approaches have been developed in an attempt to improve the blood supply for the transplanted tissue, including the application of growth factors [14, 15]. Bone marrow-derived mononuclear cells (BM-MNCs) are known to contain endothelial progenitor cells and secrete several growth factors [16]. They have also been reported to accelerate neovascularization in ischemic diseases, such as Burger's disease and ischemic heart disease, and might constitute a novel strategy for accelerating angiogenesis [17-19]. We were interested in whether BM-MNCs could facilitate neovascularization of a transplanted decellularized scaffold. The aim of the present study was to investigate the potential of the

decellularized ureter as a scaffold for constructing a tissue-engineered ureter and the role of BM-MNCs in enhancing angiogenesis in the UDM to enable the seeded UECs to survive. Below is a detailed explanation of the method.

Generation of UDM

To render the canine ureteral matrices decellular, they were excised from the beagles and treated with deoxycholic acid, as reported previously [9]. Briefly, the ureters were shaken in 10 ml of 9.1 mM PBS containing 0.1% sodium azide for 12 hours in a humidified 5% CO₂ atmosphere at 37°C. Next, the samples were treated with 10 ml 0.1 M sodium chloride containing 0.2 mg/ml deoxyribonuclease for 12 hours, followed by 10 ml PBS containing 4% sodium deoxycholate and 0.1% sodium azide for 24 hours. The treated tissues were evaluated histologically to confirm their decellular status and assess damage to the extracellular matrices. The tissues were stored in PBS with 1% antibiotic-antimycotic solution at 4°C.

Cell seeding on UDM surface

Sections (2 cm) of the UDM were perfused with 5% fibronectin in PBS for 24 hours at 4°C in order to improve cell attachment. One end of the UDM was ligated, the inner space was filled with 1×10^6 cultured UECs, and the other end of the duct was ligated. The UDM was placed in a tube with culture medium and rotated for 8 hours. The ligatures were removed and the UDM with cells was incubated in a flask for a further 3 days before transplantation.

Transplantation of UDMs with UECs: Experiment 1

As a preliminary experiment, UDMs seeded with UECs (UDM-UECs) were transplanted to the omentum of nude rats in order to investigate the fate of the seeded cells. At the time of surgery, the animals were anesthetized with sodium pentobarbital (50 mg/kg body weight injected intraperitoneally). The samples were removed and evaluated histologically at 3 days ($n = 3$) and 14 days ($n = 3$) after transplantation.

BM-MNC seeding onto UDM-UEC: Experiment 2

Bone marrow (10 ml) was aspirated from the iliac crests of dogs anesthetized with intravenous pentobarbital. BM-MNCs were isolated using a density-gradient method [19], according to the manufacturer's protocol, and suspended at 1×10^6 cells/ml in DMEM. UDM-UECs were randomly divided into two groups. In group A ($n = 3$), the UDM-UECs were implanted into the subcutaneous space of nude mice without BM-MNCs, as controls. In group B ($n = 3$), BM-MNCs (3×10^5 cells in 300 μ l) were seeded onto the outer surface of the UDM-UECs before transplantation. The grafts were removed and evaluated after 14 days. The effect of BM-MNCs on neovascularization in the grafts was assessed in two ways. First, the density of microvessels was measured by immunostaining with an antifactor VIII antibody by counting the number of microvessels in at least three fields (0.74 mm²) and calculating the mean number. Second, the distance from the inner surface of the UDM to the closest capillary was measured at 10 randomly selected points in each graft, and the mean values were calculated.

Combined effects of seeded BM-MNCs and the recipient site: Experiment 3

To investigate the effects of the recipient site on neovascularization, UDM-UECs with BM-MNCs were transplanted into the omentum of nude rats and compared with those transplanted into the subcutaneous space of nude mice. The grafts were removed after 14 days and processed for histological evaluation.

The cultured UECs began showing vacuolar degeneration 3 days after transplantation and gradually disappeared thereafter. To facilitate neovascularization in the implant, BM-MNCs were seeded around the UDM before transplantation. This facilitated the survival of the UECs, which formed three to five cellular layers after 14 days. The mean microvessel density was significantly increased in tissues seeded with BM-MNCs. However, cell-tracking experiments revealed that the increased number of capillaries in the experimental group was not due to the direct differentiation of transplanted endothelial progenitor cells. Our results demonstrate that the UDM is a useful scaffold for a tissue-engineered ureter, especially when seeded with BM-MNCs to enhance angiogenesis (Figs. 35, 36).

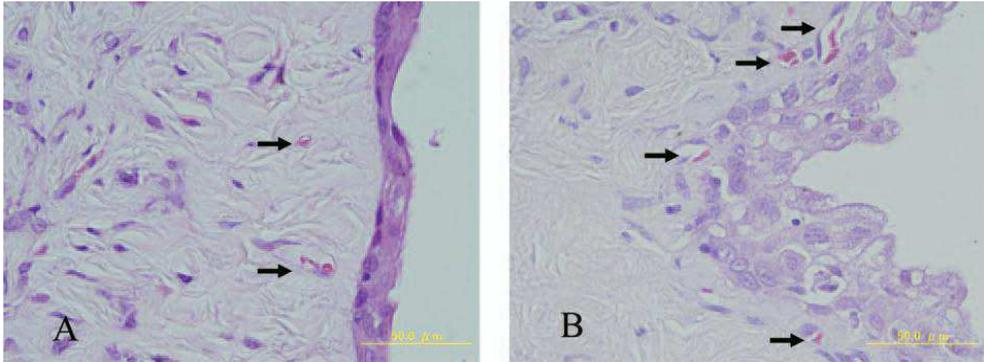


Fig. 35. Bright-field photomicrographs showing H-E staining of tissue sections from UDM-UECs transplanted with BM-MNCs to nude mouse subcutaneous space (Experiment 2) (A) and to nude rat omentum (Experiment 3) (B). Histological section from UDM-UECs with BM-MNCs implanted into the omentum exhibited a thicker epithelial cell layer than these implanted into subcutaneous space. Samples were evaluated 14 day after transplantation. In both sections, capillaries were observed close to UECs (arrows) (From Matsunuma et al. 2006. Reprinted with permission).

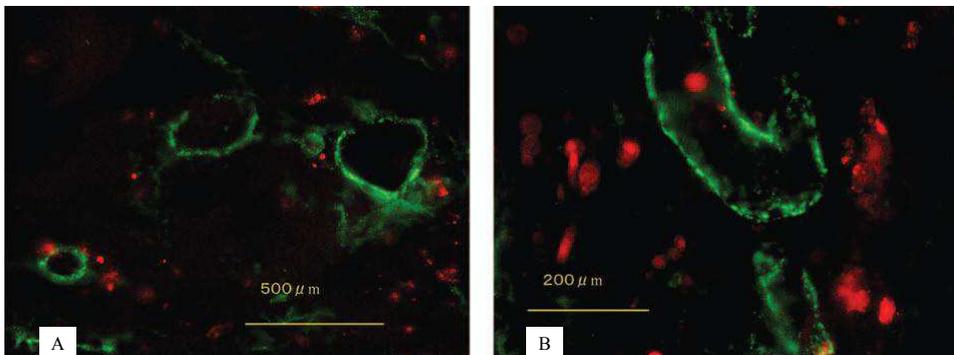


Fig. 36. Dark-field photomicrographs showing the results of cell-tracking experiments. BM-MNCs were labeled with the red fluorescent cell dye, PKH26, before being seeded onto implants, and sections of implants after 14 days were labeled for factor VIII in green using an FITC-labeled antibody. Note that only a few cells were double-labeled. A: Original magnification $\times 100$; B: $\times 200$ (From Matsunuma et al. 2006. Reprinted with permission).

This study of the initial establishment of grafts *in vivo* showed that UDMs were highly biocompatible with cultured UECs and should be a useful scaffold material for tissue-engineered ureters. Preseeding the grafts with BM-MNCs was a key factor in promoting the survival of the UECs by accelerating cell migration and neovascularization.

References

1. Pohar KS, Sheinfeld J. When is partial ureterectomy acceptable for transitional-cell carcinoma of the ureter. *J Endourol.* 15: 405,2001
2. Katz R, Golijanin D, Pode D, Shapiro A. Primary and postoperative retroperitoneal fibrosis-experience with 18 cases. *Urology.* 60: 780,2002
3. Warren JW Jr., Coomer T, Fransen H. The use of teflon grafts for replacement of ureters. *J Urol.* 89: 164,1963
4. Ulm AH. Total replacement of the human ureter with teflon prosthesis. *J Urol.* 96: 455,1966
5. Homann, W., Schreiber, B., Mlynek, M.L., Kropfl, D., and Fenkl, H.F. Long-term results of prosthetic ureteral replacement in minipigs. *Urol Int.* 39: 95,1984
6. Jonas D, Tschirkov F, Krause E. Autologous collagen graft for partial replacement of the ureter (author's translation). *Urol Int.* 28: 290,1973
7. Ahmadzadeh M. Clinical experience with subcutaneous urinary diversion: new approach using a double pigtail stent. *Br J Urol.* 67: 596,1991
8. Schaner PJ, Martin ND, Tulenko TN, Shapiro IM, Tarola NA, Leichter RF, Carabasi RA, Dimuzio PJ. Decellularized vein as a potential scaffold for vascular tissue engineering. *J Vasc Surg.* 40: 146,2004
9. Cayan S, Chermansky C, Schlote N, Sekido N, Nunes L, Dahiya R, Tanagho EA. The bladder acellular matrix graft in a rat chemical cystitis model: functional and histologic evaluation. *J Urol.* 168: 798,2002
10. Probst M, Piechota HJ, Dahiya R, Tanagho EA. Homologous bladder augmentation in dog with the bladder acellular matrix graft. *BJU Int.* 85: 362,2000
11. Elkins RC, Dawson PE, Goldstein S, Walsh SP., and Black, K.S. Decellularized human valve allografts. *Ann. Thorac. Surg.* 71: S428,2001
12. Dahms SE, Piechota HJ, Nunes L, Dahiya R, Lue TF, Tanagho EA. Free ureteral replacement in rats: regeneration of ureteral wall components in the acellular matrix graft. *Urology.* 50: 818,1997
13. Shalhav AL, Elbahnasy AM, Bercowsky E, Kovacs G, Brewer A, Maxwell KL. McDougall, E.M., and Clayman, R.V. Laparoscopic replacement of urinary tract segments using biodegradable materials in a large-animal model. *J Endourol.* 13: 241,1999
14. Kanematsu A, Yamamoto S, Noguchi T, Ozeki M, Tabata Y, Ogawa O. Bladder regeneration by bladder acellular matrix combined with sustained release of exogenous growth factor. *J Urol.* 170: 1633,2003
15. Giulian D, Woodward J, Young DG, Krebs JF, Lachman LB. Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. *J Neurosci.* 8: 2485,1988
16. Kaigler D, Krebsbach PH, Polverini PJ, Mooney DJ. Role of vascular endothelial growth factor in bone marrow stromal cell modulation of endothelial cells. *Tissue Eng.* 9: 95,2003

17. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A, Shintani S, Murohara T, Imaizumi T, Iwasaka T. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation*. 104: 1046,2001
 18. Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, Silver M, Ma H, Kearney M, Isner JM, Asahara T. Therapeutic potential of *ex vivo* expanded endothelial progenitor cells for myocardial ischemia. *Circulation*. 103: 634,2001
 19. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. *Nature*. 410: 701,2001
- (Matsunuma H, Kagami H, Narita Y, Hata K, Ono Y, Ohshima S, Ueda M)



Applied Tissue Engineering

Edited by

ISBN 978-953-307-689-8

Hard cover, 76 pages

Publisher InTech

Published online 08, June, 2011

Published in print edition June, 2011

Tissue engineering, which aims at regenerating new tissues, as well as substituting lost organs by making use of autogenic or allogenic cells in combination with biomaterials, is an emerging biomedical engineering field. There are several driving forces that presently make tissue engineering very challenging and important: 1) the limitations in biological functions of current artificial tissues and organs made from man-made materials alone, 2) the shortage of donor tissue and organs for organs transplantation, 3) recent remarkable advances in regeneration mechanisms made by molecular biologists, as well as 4) achievements in modern biotechnology for large-scale tissue culture and growth factor production.

This book was edited by collecting all the achievement performed in the laboratory of oral and maxillofacial surgery and it brings together the specific experiences of the scientific community in these experiences of our scientific community in this field as well as the clinical experiences of the most renowned experts in the fields from all over Nagoya University. The editors are especially proud of bringing together the leading biologists and material scientists together with dentist, plastic surgeons, cardiovascular surgery and doctors of all specialties from all department of the medical school of Nagoya University. Taken together, this unique collection of world-wide expert achievement and experiences represents the current spectrum of possibilities in tissue engineered substitution.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Minoru Ueda (2011). Ureter, Applied Tissue Engineering, (Ed.), ISBN: 978-953-307-689-8, InTech, Available from: <http://www.intechopen.com/books/applied-tissue-engineering/ureter>

INTECH

open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](#), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.