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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 xenogeneic 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% CO_2 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 μl) 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 mm^2) 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).

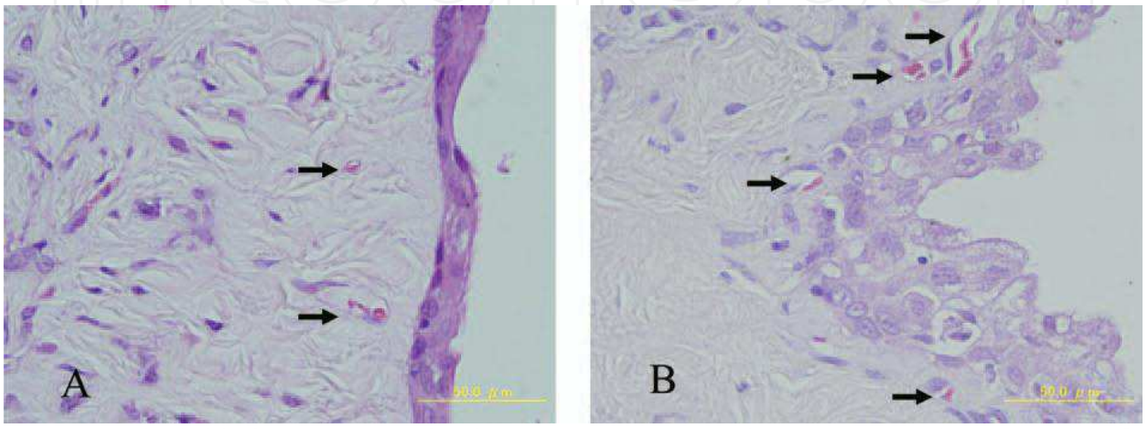

Fig. 35. Bright-field photomicrographs showing H-E staining of tissue sections from UDM-UECs transplanted with BM-MNCs to nude mouse subcutaneous space (Experiment 2) (A) and to nude rat omentum (Experiment 3) (B). Histological section from UDM-UECs with BM-MNCs implanted into the omentum exhibited a 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).

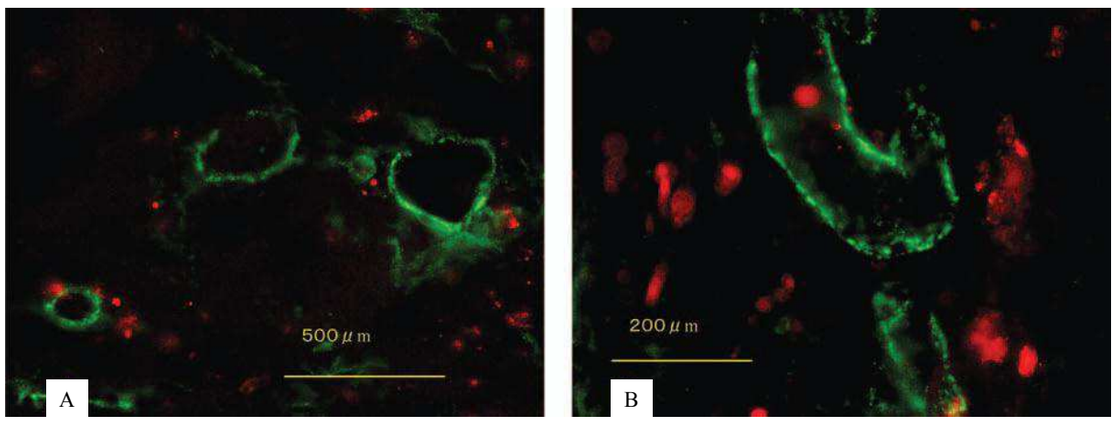

Fig. 36. Dark-field photomicrographs showing the results of cell-tracking experiments. BM-MNCs were labeled with the red fluorescent cell dye, PKH26, before being seeded onto implants, and sections of implants after 14 days were labeled for factor VIII in green using an FITC-labeled antibody. Note that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

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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.

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