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Seeding Homologous Adipose-Derived Stem Cells and Bladder Smooth Muscle Cells Into Bladder Submucosa Matrix for Reconstructing the Ureter in a Rabbit Model

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ABSTRACT

Background. Congenital or acquired abnormalities may result in ureteral malformation, trauma, or defect. Traditional reconstructive methods are often associated with numerous complications. Tissue engineering technology may provide an alternate avenue for ureteral reconstruction. In this study, we constructed tissue-engineered tubularized grafts (TETGs) by seeding homologous adipose-derived stem cells (ADSCs) and bladder smooth muscle cells (SMCs) into bladder submucosa matrix (BSM) for ureteral reconstruction in rabbit models.

Methods. ADSCs and bladder SMCs were seeded onto 2 sides of the BSM, respectively. Then the grafts were used to construct TETGs of 4.0 cm length and 8.0 mm diameter and were transplanted into the omentum of rabbits for 2 weeks before ureteral reconstruction. The 4.0-cm segment of the ureter was replaced by the TETG. Evolutionary formation of tissue structures and degree of epithelization were evaluated with the use of histologic and immunohistochemical techniques at 2, 4, 8, and 16 weeks after implantation.

Results. All of the rabbits were alive until they were killed. Histologic and immunohistochemical analyses showed consistent regeneration of mature and functional urothelium. At 16 weeks after TETG implantation, multilayered urothelium covered the entire lumen, with visible neovascularization in the center and formation of organized smooth muscle bundles.

Conclusions. We successfully constructed a tissue-engineered transplanted graft by seeding ADSCs and SMCs onto the BSM for ureteral repair and reconstruction in a rabbit model.

CONGENITAL pediatric disorders or posteriority may result in ureteral malformation, trauma, or defect. With the development of ureteroscope technology, the incidence of severe iatrogenic ureteral injuries has also increased [1]. Traditionally, surgical methods for ureteral reconstruction often required bowel replacement [2], bladder flaps [3], or kidney autotransplantation [4]. However, practical application of these reconstructive methods is associated with various limitations [5,6].

Advancements in tissue engineering have allowed successful bladder and urethral reconstruction in animal models [7,8]. For accurate regeneration of the ureter, the tissue-engineered ureter must be tubular and covered with the crucial urothelium to prevent urine from extravasating outside the surface of luminal side. At present, cell-seeded and unseeded acellular scaffolds are used for reconstructing urinary organs. Unseeded acellular scaffold is associated with a notable inflammatory response [9]; this inflammatory response is not observed with the cell-seeded acellular scaffold [10]. However, sometimes patients with malignant ureteral disease can not acquire safe urothelial cells. In such cases, stem cells can be potentially used as seeding cells for a tissue-engineered ureter. Adipose-derived stem cells (ADSCs), extensively present in the adipose tissues, can differentiate into ectodermal and endodermal cells, including epithelial cells [11–13]. Furthermore, ADSCs contain a secretome and can release several soluble growth factors that

This study was supported by the National Natural Science Funds of China (Grant No. 31400835). The first two authors contributed equally to this article.

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0041-1345/15
http://dx.doi.org/10.1016/j.transproceed.2015.10.035

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accelerate neovascularization in ischemic tissues [14]. Bladder submucosa matrix (BSM), obtained by removing cellular components, is an extracellular scaffold that is used for reconstructing tissue-engineered bladder and other organs [15]. BSM has good mechanical and physical characteristics and biocompatible properties [9]. In addition, after decellularization, the surface of BSM retains many bioactive growth factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), that promote cell growth, proliferation, and migration [16].

Deficiency of ingrowing blood vessels and inflammatory response against the transplanted material often inhibit neovascularization and result in ischemia, graft degeneration, and fibrosis [17]. Therefore, establishment of an early valid blood supply is key for graft and cell survival. The omentum has the advantage to promote neovascularization in vivo [18]. Therefore, in this study, we constructed tissue-engineered tubularized grafts (TETGs) by seeding ADSCs and bladder smooth muscle cells (SMCs) into the BSM. The TETGs were then wrapped in the omentum for 2 weeks and were transplanted into rabbits for ureteral reconstruction.

MATERIALS AND METHODS

Cell Harvesting and Culturing

ADSCs were obtained by means of collagenase digestion. Approximately 10 g of adipose tissue was carefully harvested from the inguinal region of New Zealand white rabbits (weight, ∼2–2.5 kg). After being washed 3 times with 10% neomycin sulfate, the adipose tissue was minced thoroughly, transferred to 0.1% type I collagenase (Sigma-Aldrich, St Louis, Missouri), and incubated for 1 hour at 37°C in an incubator. The solution containing the adipose tissue was centrifuged at 2,000 rpm for 10 minutes, and the cell pellet was resuspended in Dulbecco modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 150 U/mL penicillin, and 150 U/mL streptomycin. The obtained cells were inoculated in cell culture plates (diameter, 10 cm) and were incubated at 37°C under 5% CO_2. Passage 3–5 ADSCs were used for the next step.

SMCs were obtained from bladder biopsies of New Zealand white rabbits with the use of a tissue explant technique. Briefly, 1 × 1-cm muscle tissue was excised from the bladder wall. Primary cultures of SMCs were obtained by explanting muscle tissues in cell culture plates (diameter, 10 cm) containing DMEM supplemented with 10% FBS and incubating at 37°C in 5% CO_2. Culture medium was changed every 3 days. After 7 days, the growing cells were trypsinized and subcultured, and passage 3–5 SMCs were used for the next step.

Identification of ADSCs and SMCs

Flow cytometry was performed to confirm phenotypic stem cell markers on ADSCs obtained after culturing to near confluence. Cell density, as determined with the use of a hemocytometer, was adjusted to 2×10^6 cells/mL. The resuspended cells were incubated with respective monoclonal mouse antirabbit antibodies to detect CD34, CD44, CD45, and CD90. The reaction of the antibodies with CD34, CD44, CD45, and CD90 was determined with the use of a flow cytometer (BD Biosciences, Franklin Lakes, New Jersey), and the samples were analyzed with the use of Cell Quest software (BD Biosciences). SMC phenotype was identified by means of immunofluorescence for α-smooth muscle actin (α-SMA).

Preparation of BSM

Fresh bladders taken from New Zealand white rabbits were rinsed with phosphate-buffered saline solution (PBS) and transferred to PBS supplemented with 0.1% sodium azide. Bladder segments were placed on a rocker to stir for 24 hours. Then bladder tissues were decontaminated with the use of 100 mL PBS, incubated with 1 mol/L sodium chloride containing 2,000 Kunitz units deoxyribonuclease I (Sigma, United Kingdom), and stirred at 37°C for 10 hours. After lysis, all of the intracellular constituents were released. The bladder materials were then incubated with 4% sodium deoxycholate containing 0.1% sodium azide at room temperature for 24 hours with stirring to solubilize lipid components of cells. This process was repeated twice. The harvested BSM was washed three timeswith PBS and preserved at 4°C in penicillin-streptomycin solution. Hematoxylin and eosin (HE) staining, Masson trichrome staining, Sirius red in saturated carbazotic acid staining, and scanning electron microscopy (SEM) were performed to assess the complete process of BSM and degree of acellularity.

Seeding Cells onto the BSM to Produce TETGs

Passage 3–5 ADSCs and SMCs were resuspended (2×10^7 cells/mL) and inoculated onto the BSM. The BSM was sequentially inoculated with ADSCs within the lumen and incubated for 3 days, and with SMCs on the outer surface and incubated for 3 days. The compound matrix was cultured for 1 week, and the medium was refreshed daily. Next, the compound matrix was assessed with the use of HE staining and SEM. With ADSCs on the luminal side, the 4-cm compound matrix was wrapped on a catheter (diameter, 8.0 mm) to produce TETGs. Then the TETGs were wrapped in the omentum obtained from New Zealand white rabbits and incubated for 2 weeks until reconstitution of the ureter was initiated. HE staining and immunohistochemical analysis of transplants were performed to detect epithelial regeneration and neovascularization. Unseeded BSM also was wrapped in the omentum and as a control group. Only one side seeded with ADSCs and only the other side seeded with SMCs served as 2 partial control groups.

Reconstitution of the Ureter with Cell-Seeded TETGs

In all, 20 male New Zealand white rabbits (weight, 2.5–3.0 kg) were divided into 4 groups. The animals were anesthetized with the use of pentobarbital sodium (30 mg/kg) before operation. The unilateral ureter was exposed and identified through a small ventral midline abdominal incision, and a ∼4.0-cm segment of ureter removed, which was replaced over the ureteral defect by the TETGs. Anastomotic sites were repaired with the use of 6–0 absorbable sutures. Urine was guided with an indwelling ureteral catheter for 6 weeks after the operation.

Histologic and Immunohistochemical Analyses

In all, 1 rabbit from each group was killed at 2, 4, 8, and 16 weeks after implantation. Histologic evaluation of the transplanted matrix was performed at 2, 4, 8, and 16 weeks after implantation. After removal from the rabbits, each harvested graft was fixed in 10% formalin for ≥24 hours, placed in paraffin wax, and serially sectioned. For histologic analysis, conventional HE staining and Masson trichrome staining were performed. For immunohistochemical analysis, regeneration of epidermal cell layers from the graft was identified with the use of mouse anti–rabbit AE1/AE3,

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