Journal of Pediatric Surgery (2006) 41, 2090–2094

ELSEVIER

Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

A comparison of engineered urinary bladder and intestinal smooth muscle for urinary bladder wall replacement in a rabbit model☆

Jin-Yao Lai MD*, Pei-Yeh Chang MD, Jer-Nan Lin MD

Department of Pediatric Surgery, Chang-Gung Children's Hospital, School of Medicine, Chang-Gung University, Kweishan, Taoyuan 333, Taiwan

Index words:
Urinary bladder;
Small intestine;
Polyglycolic acid;
Cell culture;
Bladder augmentation

Abstract

Background/Purpose: The small intestine is the most common resource for bladder augmentation. Little is known whether intestinal smooth muscle cells (SMCs) may be engineered into bladder tissue. We investigated the phenotypic and functional characteristics of engineered bladder and intestinal SMCs as bladder wall replacement in a rabbit model.

Methods: One month after an initial 70% partial cystectomy, 3 autoaugmentation surgeries were performed, including traditional autoaugmentation (TA, n = 6), TA using engineered bladder SMCs (TA + B, n = 6), and TA using intestinal SMCs (TA + I, n = 6). All were followed up by bladder volume measurement and retrieved on the first, third, and sixth month. The grafts and the native bladder wall were evaluated with immunocytochemistry and electrical field stimulation (EFS). Statistical analysis was performed using analysis of variance.

Results: Both the TA + I and TA + B groups showed significant and similar bladder capacity increment in all time points. The engineered muscle cells demonstrated the typical "contraction-relaxation" response to supramaximal EFS. There were no statistical differences in both the TA + I and TA + B groups in contractility force.

Conclusion: Engineered SMCs derived from urinary bladder and small intestine could retain their phenotype after implantation in vivo. Both exhibited a similar degree of contractility to EFS. These results suggest that there are no phenotypic or functional differences between muscle cells obtained from the 2 different organs. Both have the potential to be engineered into normal bladder tissues.

Conventional enterocystoplasty is associated with a variety of complications [1-3], which in turn stimulated the development of alternative methods for bladder augmentation. Autoaugmentation, which preserves intact urothelial layers, prevents the complications related to the intestinal mucosa. However, the bladder volume increment after the autoaugmentation is often limited without a satisfactory bladder wall support [4-7]. Autoaugmentation with

Presented at the 39th Annual Meeting of the Pacific Association of Pediatric Surgeons, May 14–18, 2006, Taipei, Taiwan.

☆ Supported by grants from the National Science Council of the Republic of China (No. NSC90-2314-B-182A-024) and from Chang Gung Memorial Hospital (CMRP 1296).

* Corresponding author. Tel.: +886 3 328 1200x8227; fax: +886 3 328 7261.

E-mail address: jylai@cgmh.org.tw (J.-Y. Lai).

0022-3468/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jpedsurg.2006.08.013

demucosalized seromuscular colonic or gastric flaps offers good bladder wall support. These flaps prevent adhesions, shrinkage, perforation, and even leakage of the protruded mucosa. Disadvantages of these flaps include operative time, technical difficulties, and postoperative complications [8,9].

We have demonstrated that engineered bladder smooth muscle cells (B-SMCs) could be an effective bladder wall support during bladder autoaugmentation in a rabbit model [10]. Clinically, the small intestine is the most common resource for bladder augmentation. It is not known whether isolated intestinal SMCs (I-SMCs) may be engineered into a functional muscular tissue. We attempted to determine the phenotypic and functional characteristics of the engineered B-SMCs and I-SMCs as bladder wall replacement in a rabbit model.

1. Materials and methods

1.1. Polyglycolic acid scaffolds

Unwoven sheets of polyglycolic acid polymers (Smith and Nephew, Heslington, York, UK) were trimmed to 4 × 4-cm patches. The scaffolds were designed to degrade via hydrolysis during a 4-week period. The scaffolds were coated with a liquefied copolymer PLGA (Sigma Chemical Co, St Louis, Mo), 80 mg/mL, to achieve adequate mechanical characteristics. The polymers were sterilized in ethylene oxide and stored under sterile condition until used.

1.2. Animals

Experiments were performed on male New Zealand white rabbits with body weight of about 3 kg. This project was approved by the Animal Research and Care Committee at Chang-Gung Children's Hospital. All animals were in good health.

1.3. Bladder SMC seeding and implantation

All rabbits received 70% partial cystectomy (PC). Bladder specimens were obtained during PC. The B-SMCs were cultured by using a previously described technique [10,11]. The muscle cells were expanded until the desired cell numbers were obtained.

1.4. Small I-SMC seeding and implantation

A piece of the small intestinal wall, about 2 × 2 cm in size, was obtained at the same time. The muscle layer was stripped from the mucosa. The I-SMCs were cultured by the same technique used with B-SMCs. The muscle cells were expanded until the desired cell numbers were obtained. The seeding density was the same as with the B-SMCs.

1.5. Bladder autoaugmentation

One month after PC, all rabbits were divided into 3 groups. A 4 × 4-cm muscular defect was created at the anterior bladder surface without perforation into the mucosal layer. In the group using traditional autoaugmentation (TA, n = 6), the bulging mucosa was left uncovered. In TA + B (B-SMCs, n = 6) and TA + I (I-SMCs, n = 6), the cell-seeded scaffolds were patched to the prolapsed mucosa, respectively. Perivesical fat was used to cover the implanted grafts as a source of blood supply. After the operation, the rabbits were allowed to resume normal diet. Bladder volume measurements were performed in all animals on 1, 3, and 6 months time point. At the time of harvest, gross appearance of the grafts was identified by the silk stitches that were put during surgery.

1.6. Bladder volume measurements

Bladder volume measurements were performed by using a 7-F double-lumen transurethral catheter. The bladder was emptied and the intravesical pressure was recorded as resting pressure. The pressure was then recorded during instillation of prewarmed saline solution at constant rates until the pressure of 40 cm H_2O was reached. The bladder capacity at 40 cm H_2O was used as "bladder volume."

1.7. Histologic and immunocytochemical analyses

The scaffolds were placed in 10% neutral buffered formalin for histologic (hematoxylin and eosin) and immunohistochemical evaluation (smooth muscle α-actin and pancytokeratin AE1/AE3). We used 3,3′-diaminobenzidine tetrahydrochloride as the enzyme substrate, which yielded granular brown deposits for positive interpretation.

Table 1 The changes in bladder volume in percentage

	Control	TA	TA + B	TA + I
Preoperative	100	100	100	100
Partial cystectomy	27.94 ± 14.05	34.71 ± 4.51	35.51 ± 5.16	35.12 ± 5.01
First month	35.50 ± 5.41	37.74 ± 4.75	54.03 ± 1.12*	51.30 ± 2.79*
Third month	37.52 ± 6.48	42.62 ± 8.35	73.25 ± 3.71*	71.42 ± 4.81*
Sixth month	36.55 ± 0.42	41.14 ± 1.25	76.48 ± 3.10*	76.91 ± 3.65*

* Indicates $P < .05$ as compared with the control group.