The bladder submucosa acellular matrix as a cell deliverer in tissue engineering

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Background: **This study was designed to explore the possible use of the bladder submucosa acellular matrix (BSAM) as a cell deliverer in tissue engineering**.

Methods: **Smooth muscle cells (SMCs) were isolated from bladder tissues by collagenase digestion and cultured and passaged in DMEM supplemented with 10% fetal bovine serum. Bladder submucosa was extracted from bladder by microdissection and washed thoroughly with 0.5% SDS and dH_2O. Smooth muscle cells were seeded onto the matrix at a density of 5.0×10^6 cells per cm^2. The cell-matrix complex was harvested at 1, 2, 3 and 4 weeks. The growth of SMCs was evaluated by HE staining and electronic microscopy**.

Results: **SMCs adhered to the BSAM. One week later they located on the limited surface areas of the matrix or penetrated into the surfaces. After cell seeding for 2 and 3 weeks, the quantity of the cells increased markedly and most of them reached the inner side of the matrix. Four weeks after seeding, however, the number of the cells in the cell-matrix complex decreased**.

Conclusions: **The BSAM as a well biocompatible material *in vitro* can be used as a cell deliverer in tissue engineering research. The BSAM seeded with cells should be placed back to the host within 3 weeks after cell seeding.**

World J Pediatr 2006;1:57-60

Key words: **bladder; cell; culture; acellular matrix; deliverer**

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Introduction

Tissue engineering including the use of matrices for tissue regeneration and selective cells for transplantation has provided a new approach to create functional organs or tissue segments. Some reports have focused on clinical application of tissue reconstruction in a variety of disorders, such as engineered injectable chondrocytes for the correction of vesicoureteral reflux (VUR) and urinary incontinence, and the collagen-based matrices for urethral reconstruction.[1-4]

Acellular matrix, which is mainly made of the supporting connective tissue, has been widely studied as a biodegradable scaffold for tissue regeneration. Merguerian et al[5] directly used bladder acellular matrix graft (BAMG) to repair bladder defect in porcine model. At 16 days the luminal surface was lined with a single layer of the urothelium. At 30 days the urothelium was multilayered with organized groups of smooth muscle cells (SMCs) and angiogenesis. Kropp et al[6] determined the feasibility of urinary bladder regeneration by porcine-derived small intestinal submucosa (SIS) in SD rats which underwent partial cystectomy and bladder augmentation. At last, the induced bladder tissue contained all 3 layers of the normal bladder, and was functionally contractile. Both studies, in which the acellular matrix was used to repair tissue defect directly, have shown that the acellular matrix could induce specific tissue regeneration *in vivo*. However, experimental studies showed that these matrices with no cells implanted in the bladder contract with time and lead to diminished bladder capacity.

The bladder submucosa acellular matrix (BSAM) is a xenogenic, collagen rich membrane. In this study, we evaluated *in vitro* the growth state of bladder SMCs seeded on the BSAM in order to demonstrate the characteristics of reaction between bladder smooth cells and the BSAM, and the possible use of the BSAM as a cell deliverer in tissue engineering.

Methods

Culture and expansion of bladder SMC

Bladders were obtained from Changfeng white pork.

Under sterile conditions, serosa was dissected, and the urothelium was scratched off. The remaining smooth muscle layer was then minced into small pieces of 0.1 mm × 0.2 mm and digested in 0.1% collagenase II for 6 hours. Cell suspensions of individual smooth muscle were washed with phosphate buffered saline, suspended, and placed in cell culture flasks (75 cm^2). Culture medium DMEM was supplemented with 10% fetal bovine serum. The cells were incubated at 37℃ in a humidified atmosphere of 5% CO_2 in air. The medium was changed twice a week. Once the cells in the flasks reached a 90% confluence, the medium was removed and 2 ml of trypsin-EDTA (0.05% and 0.02%) was added for 5 minutes. As soon as phase contrast microscopy confirmed that cell detachment had occurred, the digestion was discontinued. The passage ratio was 1∶4.

Preparation of BSAM

After the whole bladder had been harvested from pork, it was rinsed with phosphate buffered saline. The submucosa was microdissected off the muscular and serosa layers. It was thoroughly washed in 5% SDS and dH_2O to become a matrix, which was subsequently trimmed into 2 cm × 3 cm pieces, lyophilized, sterilized with ethylene oxide, and stored for use. Random samples of the matrix were obtained for evaluation.

Seeding of bladder SMC onto BSAM

Passaged for 3 or 4 times, SMCs had been in sufficient number. They were seeded onto the BSAM at a concentration of 5.0×10^6 cells per cm^2. Later on they were left in air for approximately 3 to 4 hours to adhere to the matrix. Ten ml DMEM supplemented with 10% fetal bovine serum was carefully pipetted down to the cell-matrix complexes. All cultures were incubated at 37℃ in a humidified atmosphere of 5% CO_2 in air. Once the cells were seeded onto the BSAM, the medium was changed daily. These cell-matrix complexes were harvested at 1, 2, 3 and 4 weeks after seeding.

Histology and electronic microscopy

Smooth muscle cell specific protein (α-actin) was identified by the immunohistochemical method. The BSAM and the growth of smooth muscle cells on it were evaluated by HE staining, Masson trichrome, and electronic microscopy.

Results

Culture of bladder smooth muscle cell

Porcine bladder smooth muscle cells displayed a spindle-shaped feature with a centrally located nucleus. Moreover, cell nodules formed a typical structure of "peaks and valleys". Immunohistochemical staining with an α-actin antibody specific for smooth muscle confirmed that these cells contained this characteristic antigen. The cells expanded rapidly without marked decrease in growth rate even after 4 passages.

BSAM

The BSAM was white, semi-translucent and approximately 0.1 to 0.2 mm thick. Masson trichrome staining showed that the matrix was collagen rich membrane. HE staining and electronic microscopy did not find any cells or the remained cellular elements.

Cell seeding

Electronic microscopy showed that smooth muscle cells extended on the surface of the BSAM and adhered to it, and lots of minivilli were seen on the cell membrane (Fig. 1). One week after seeding, a majority of smooth muscle cells were confined to the matrix surface. Only in the limited areas, the cells penetrated through the surface of the submucosa matrix (Fig. 2). From 2 to 3 weeks after cell seeding, the quantity of the cells increased markedly and many of them penetrated through the acellular matrix surface (Fig. 3). By the end of 4 weeks, the quantity of the cells in the matrix decreased markedly and they just located on the surface of the acellular matrix. During the whole course, even 4 weeks after seeding, the cell-matrix complex showed spindle-shaped cells emanating from the tissue of smooth muscle.

Discussion

Gastrointestinal segments are commonly used as a tissue for replacement or reconstruction of the bladder and ureter. However, when gastrointestinal tissue is in

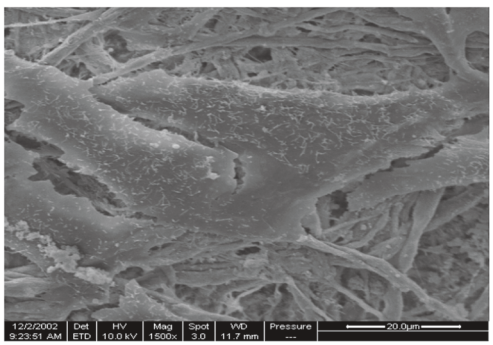

Fig. 1. Electronic microscopy showing smooth muscle cells adhering to the BSAM and abundant minivilli on the cell membrane.

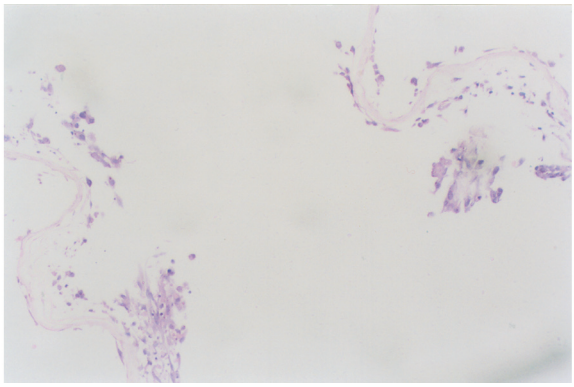

Fig. 2. One week after cell seeding, a majority of smooth muscle cells confined to the submucosa surface in limited areas of the matrix (HE staining, original magnification ×40).

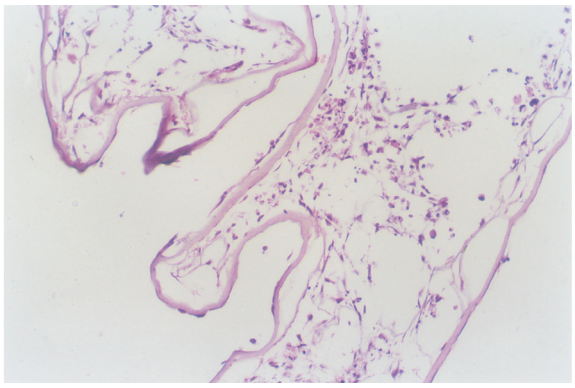

Fig. 3. Smooth muscle cells penetrated into the BSAM and expanded for 2 weeks (HE staining, original magnification ×100).

contact with urine, many complications occur, including infection, metabolic disturbance, urolithiasis, perforation, increased mucus production, and tumor development.[7-11] Hence, numerous investigators have attempted to use alternative materials and tissues for reconstruction of the bladder and ureter.[12,13]

Presently, both cell-seeded and unseeded types of tissue engineering production are being tried in reconstruction of the urinary tract.[14] Cell-unseeded techniques involve direct *in vivo* placement of a cell-unseeded biodegradable material that will function as a scaffold to allow the cells that constitute the tissue being replaced to grow inside and form the tissue structure. Cell-seeded techniques need to seed biodegradable materials *in vitro* the culturally expanded cells that are harvested from the host tissue and to form the complex patch.[15] This composite structure will be placed back to the host to continue the regenerative process and finish the replacement. During this method, the biomaterial used as cell delivery vehicle is one of the key structures. Except biomaterial and its degradation products are required to be nontoxic to the cells and host, they also should be biocompatible since the growth of new tissue strongly depends on the interaction between the biomaterial and seeded cells. Presently various synthetic biomaterials[16] including polyglycolic acid (PGA), polylactic acid (PLA) and polylacticglycolic acid (PLGA) have been used experimentally with excellent biocompatibility. Oberpenning and colleagues[17] reported that beagle dog's bladder was replaced by tissue engineered bladder, in which synthetic material PGA was used as a cell deliverer. But these synthetic materials also have some shortcomings, like expensiveness, lower elasticity, and unsuitability for surgical suture. [18]

Thus the collagen-based material derived from the small intestine and bladder has been taken as a cell deliverer. Since this naturally derived matrix is obtained from normal tissue, it provides an environment and framework that are more conducive to the regeneration process than that of synthetic material. And they contain numerous factors that are likely to be critical to the induction of tissue regeneration.[19]

The BSAM is naturally derived from donor porcine bladder. Chen et al[20] used this material to replace urethral defect in an rabbit model. Gross examination showed normal appearance of the tissue without evidence of fibrosis. Histologically, the implanted matrices showed host cell infiltration and angiogenesis 2 weeks after surgery. Immunohistochemical staining demonstrated the migration of unorganized muscle fiber bundles 2 months after implantation and organized muscle bundles 6 months after implantation. It was also demonstrated that the BSAM without cells, when used for bladder augmentation, was able to assimilate itself histologically to the native bladder, but contracted over time.[21] Both reports showed that the BSAM can promote tissue regeneration and that it is a biodegradable, well biocompatible, and nontoxic biomaterial *in vivo*.[22,23]

In the present study, the BSAM was evaluated in a cell-seeded manner *in vitro*, which enhanced its ability to accomplish the regeneration of the bladder or ureter, which was superior to that induced by the unseeded BSAM. Bladder smooth muscle cells were found to adhere to the BSAM, penetrate into the matrix, and expand in it for 3 weeks after cell seeding. This finding reveals that the BSAM can be used as a cell deliverer in tissue engineering research.[24,25]

In conclusion, the BSAM as a well biocompatible material is nontoxic to cells *in vitro*. It can be used as a cell deliverer in tissue engineering research. The BSAM seeded with cells should be placed back into the host within 3 weeks after cell seeding.

Funding: This study was supported by the grants from the Chinese Natural Science Foundation of China (301709361), and Shanghai

"Shu Guang Ji Hua" of the Shanghai Educational Committee Foundation (99ZD06), Shanghai, China.

Ethical approval: This study was approved by the data inspectorate of China and by the regional committee for medical research ethics.

Competing interest: None declared.

Contributors: GHQ proposed the study and wrote the first draft. All authors contributed to the design and interpretation of the study and to further drafts. CF is the guarantor.

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Received April 26, 2005
Accepted after revision December 30,2005