Bladder Acellular Matrix Conjugated with Basic Fibroblast Growth Factor for Bladder Regeneration

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Basic fibroblast growth factor (bFGF) plays an important role in wound repair and tissue regeneration. Considerable research has been focused on the exploration of bFGF delivery systems for maintaining efficient local concentration at the injury sites. In this study, bFGF was chemically crosslinked to the bladder acellular matrix (BAM) to create specific binding between bFGF and BAM. The BAM scaffold conjugated with bFGF (CL-BAM/bFGF) could bind more bFGF and achieve controlled release of bFGF, which promoted human fibroblasts to proliferate *in vitro* and accelerated cellularization and vascularization after subcutaneous implantation. Using the rat bladder reconstruction model, the CL-BAM/bFGF group showed better tissue regeneration compared with the other groups. In summary, CL-BAM/bFGF could prevent the diffusion of bFGF from BAM and maintain its activity. Thus, the scaffold conjugated with growth factor systems could be an effective way for maintaining local therapy dosage at the target site in wound repair and tissue regeneration.

Introduction

DISORDERS, INCLUDING CONGENITAL abnormalities, cancer, or other serious bladder disease, can lead to bladder damage or loss of function. Most of these cases require the surgical removal of the bladder, and gastrointestinal segments are commonly used materials for bladder reconstruction. However, gastrointestinal tissues can absorb and excrete specific solutes, thus many complications may occur, such as infection, metabolic disturbances, urolithiasis, increased mucus production, and malignancy.^{1–3} Hence, it is necessary to find alternative materials to improve the current method for bladder reconstruction.

Currently, a number of biomaterials have been used in the study of bladder reconstruction.^{4,5} Bladder acellular matrix (BAM) has been widely used in the reconstruction of bladder in various models.⁶ As derived from native bladder, BAM retains some active growth factors, and its natural extracellular matrix (ECM) structure was favorable for bladder cells to recognize and grow in.^{7,8} However, after removal of cellular components, the amount of these residual bioactive factors left in the BAM may not be sufficient.⁹ For repair of a severely damaged bladder, BAM alone is insufficient to get desirable results.¹⁰ Novel composite biomaterials activated by bioactive factors have drawn much attention for effective tissue regeneration.¹¹

The basic fibroblast growth factor (bFGF) plays an important role in tissue regeneration.^{12–14} Previous studies have

shown that BAM absorbed with bFGF can promote angiogenesis and smooth muscle regeneration.⁸ However, the binding of bFGF on biomaterials is difficult because of its rapid diffusion in body fluids, which leads to low therapeutic concentrations at the target site and unfavorable side effects to the body.¹⁵ Therefore, constructing an efficient delivery system that can retain growth factors at the target site is very important.¹⁶

In the present study, bFGF was immobilized on BAM by a chemically covalent bond using the Traut's reagent and the SMCC reagent. It is hypothesized that the BAM scaffold conjugated with bFGF will be an effective approach for bladder regeneration.

Materials and Methods

Preparation of BAM

All animal procedures were approved by the Chinese Ministry of Public Health guidelines. BAM was prepared according to the protocols published previously.⁷ Briefly, whole bladders were harvested from 25 to 30 kg pigs under sterile conditions and incubated in a hypotonic solution consisting of 10 mM Tris HCl (pH 8.0), 5 mM ethylenediaminetetraacetic acid (EDTA; Amersco), and 10 KIU/mL aprotinin (Livzon Pharmaceutical Group, Inc.) at 4°C for 48 h. Then, the bladder was incubated in 1.0% Triton X-100 (Sigma-Aldrich) at 4°C for 48 h, then treated with 50 U/mL DNase I and 1 U/mL RNase A (Sigma-Aldrich) for another

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24 h at 4°C. Finally, the bladders were rinsed with a tris-buffered sodium dodecyl sulfate (SDS) solution at 4°C for 48 h. After washing with double-distilled water, the BAM was frozen, lyophilized, sterilized by 12 kGy Co⁶⁰ irradiation, and sealed at -20°C until use. For the histological examinations, the sections were stained with hematoxylin and eosin (H&E) and immunofluorescence analysis using anti-collagen I (1:20; Southern Biotech), anti-collagen IV (1:20; Southern Biotech), anti-fibronectin (1:200; Santa Cruz), and anti-laminin (1:50; Sigma-Aldrich).

Introduce sulfhydryl groups

Traut's reagent (2-iminothiolane or 2-IT) (Pierce) is a cyclic thioimide compound for sulfhydryl addition. Once added, sulfhydryl groups can be specifically targeted for chemical modifications. For analyzing the efficiency of Traut's reagent to introduce sulfhydryl groups on BAM, different concentrations of Traut's reagent (10, 5, 2.5, 1.25, 0.63, and 0.31 mg/mL) were dissolved in phosphate-buffered saline (PBS) with 4 mM EDTA. The BAM scaffold was soaked in Traut's reagent solution at 4°C for 12 h. After washing, the BAM scaffold was immersed in 0.1 mg/mL Ellman's reagent (Sigma-Aldrich) to react with sulfhydryl groups for 15 min at room temperature (RT), and then the samples were analyzed with an ELISA reader (Tecan Sunrise) at 405 nm.

Conjugating bFGF

Succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) (Pierce) is an amine-to-sulfhydryl cross-linker, which contains an amine-reactive N-hydroxysuccinimide (NHS ester) and a sulfhydryl-reactive maleimide group. In this study, the NHS ester was reacted with the bFGF, excess SMCC removed, and then the sulfhydryl-containing BAM was added. This two-step reaction scheme resulted in the formation of specific binding of BAM/bFGF conjugates. To investigate if the SMCC concentration affected the conjugation efficiency, different concentrations of SMCC, 5-, 10-, 20-, 40-, 60-, or 80-fold molar excess to the bFGF, were tested in the conjugation reaction. SMCC was reacted with bFGF at RT for 1 h. Then, sulfhydryl-containing BAM was added at RT for 2 h. After incubation, the scaffolds were washed three times in PBS on a shaker to remove nonbound bFGF and the extra chemical agent.

Morphological observation

The morphology of BAM and CL-BAM (crosslinked BAM with Traut's reagent and SMCC) was observed with a scanning electron microscope (SEM, S-3000N; Hitachi). The samples were dried by a freeze dryer, coated with gold on an ion sputterer (E-1010; Hitachi), and then viewed by SEM at a voltage of 15 kV.

Binding ability

bFGF with increasing concentrations from 0.5 to 8 µM was added onto the BAM and CL-BAM group. Then, the scaffolds were washed five times by 100 µL of PBS to remove the unbounded bFGF. The amount of bFGF bound to the BAM scaffolds was quantified by an ELISA assay.

Briefly, the scaffolds were blocked with bovine serum albumin (BSA) (50 mg/mL) in PBS for 1 h. After washing with PBS plus 0.05% Tween-20 (PBST), the scaffolds were then incubated with the mouse anti-bFGF antibody (Sigma-Aldrich; 1:400) for 1 h at RT. After washing three times with PBST (5 min each) on a shaker, the anti-mouse alkaline phosphatase antibody (Sigma-Aldrich; 1:10,000) was added at RT for 1 h, followed by washing three times with PBST as above. Samples were detected with p-nitrophenyl phosphate hexahydrate (p-NPP) (Amersco) for 15 min and measured at 405 nm in an ELISA reader.

Controlled release

After conjugation with bFGF (10 µg), the BAM group and the CL-BAM group were soaked into a releasing buffer (PBS with 0.02% sodium azide) for 0, 12, 24, 48, 72, and 96 h at RT with continuous shaking. The buffer was changed into a fresh one every 12 h. The remained bFGF on scaffolds was analyzed by the ELISA as mentioned above.

The bioactivity in vitro

Each scaffold (10 µg bFGF) was put into 96-well plates and washed three times with PBS. Then, the human fibroblasts were seeded at a density of 5000 cells per well and cultured in the DMEM (Hyclone) supplemented with 2% fetal bovine serum. The culture medium was replaced every 2 days. After culture for 5 days, the scaffolds were washed with PBS for three times and incubated for 1 min at RT with 5 mg/mL fluorescein diacetate (FDA; Sigma). After washing with PBS, samples were evaluated by a confocal microscope (Leica Microsystems). To investigate the proliferation of the cells, the samples were taken out on days 1, 3, 5, and 7. Cell proliferation assay was determined by the methylthiazol tetrazolium assay (MTT) method.

Subcutaneous implantation

Twelve male Sprague Dawley rats 200 ± 10 g were randomly divided into four groups: group 1 (BAM group) was BAM; group 2 (BAM/bFGF group) was BAM scaffold simply adsorbed with bFGF (10 µg); group 3 (CL-BAM group) was BAM crosslinked with Traut's reagent and SMCC; group 4 (CL-BAM/bFGF group) was BAM scaffold crosslinked with bFGF (10 µg) using Traut's reagent and SMCC. Animals were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg). The middle back area was shaved and disinfected with 75% alcohol and iodophor. Four 1 cm incisions were made on the dorsum of each rat, and then the scaffolds from the four groups were randomly embedded into the four subcutaneous pockets in one rat. The incisions were then closed with interrupted sutures, followed by intramuscular injection of gentamycin (5 mg/kg) for 3 days continuously. At day 14 postsurgery, the rats were euthanized. Sections were made for H&E staining and immunohistochemical staining with the anti-von Willbrand factor antibody (vWF, 1:800; Abcam).

Bladder regeneration

Thirty male Sprague Dawley rats (300 ± 10g) were randomly divided into five groups: BAM group, BAM/bFGF (10 µg) group, CL-BAM group, CL-BAM/bFGF (10 µg) group, and sham-operated group. Animals were anesthetized as mentioned

FIG. 1. Hematoxylin and eosin (H&E) and immunofluorescence staining of normal bladder (top panel) and decellularized bladder (bottom panel). (A, F) H&E staining; (B–E, G–J) immunofluorescence staining. Scale bar = 100 μ m. Color images available online at www.liebertpub.com/tea

above. A bladder regeneration model was made as previously described.¹⁷ The upper half of the bladder (dome and upper part) was excised. The scaffolds with a diameter of about 1 cm were grafted to the resultant defect using a two-layer closure with 5.0 vicryl sutures. After operation, the rats were housed in separate cages according to the standard protocol.

Urodynamic study

The urodynamic study was performed at day 90 after surgery. After anesthetization, the rats were placed on a 37°C thermostat plate. A lower midline abdominal incision was made to expose the bladder. A catheter was inserted into the bladder and connected to a pressure transducer with a syringe pump infusing physiological saline at the rate of 0.1 mL per minute. The urodynamic signals were acquired by the Pulab-UE system and the following parameters were evaluated: bladder volume capacity, maximum intravesical pressure, and bladder compliance.

Mechanical test

The samples from the reconstruction bladder were cut into 0.5 $\times$ 1.5 cm size and measured by a tensile testing machine (Tinius Olsen). All measurements were made within 4 h after sacrifice. The test speed was 2 cm/min. Ultimate tensile strength was calculated from the stress–strain curves.

Histology analysis

At day 90 after surgery, sections were made and stained with H&E. Immunostaining was performed to observe the bladder regeneration and neovascularization with the anti- α -smooth muscle actin antibody (α -SMA, 1:100; Abcam) and the anti-von Willebrand factor antibody (vWF, 1:800; Abcam). Image-Pro Plus software was used for quantification as follows: Percentage of α -SMA-positive area = α -SMA-positive area/Total tissue area and the number of capillary vessels per field was also counted.

Statistical analysis

All data are expressed as mean $\pm$ standard deviation (SD), and statistical analyses were performed with Statistics Package for Social Science (SPSS) software. Any significant

differences in the binding assay, controlled release assay, and bioactivity assay *in vitro* were determined by a two-tail *t*-test. The subcutaneous implantation assay and the bladder regeneration assay were determined by one-way analysis of variance (ANOVA), while multiple comparisons between two groups were determined by the Tukey's Multiple Comparison Test. Statistically significant difference was determined by *p*-values at $*p < 0.05$ and $**p < 0.01$.

FIG. 2. The quantifications of Traut's reagent and succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) in crosslinking reactions with bladder acellular matrix (BAM). (A) The quantifications of Traut's reagent. Initial concentrations of Traut's were 10, 5, 2.5, 1.25, 0.63, and 0.31 mg/mL, respectively. (B) The quantifications of SMCC. Initial fold molar excess of SMCC was 80, 60, 40, 20, 10, and 5, respectively. Data are presented as mean $\pm$ SD.

FIG. 3. Scaffold morphology by SEM. (A) The morphology of BAM under 100 magnification. (B) The morphology of CL-BAM under 100 magnification. Scale bar = 500 μ m.

Results

The properties of BAM

The H&E results revealed that the homogeneous pink staining was typical of collagen in the biomatrix, whereas the basophilic staining typical of cellular nuclear material was not observed (Fig. 1F). The immunostaining for the four ECM proteins, collagen I, collagen IV, fibronectin, and laminin, indicated that both the ECM structure and basement membrane components of the ECM were preserved (Fig. 1G–J) and were similar to the native bladder (Fig. 1B–E). The lack of PI staining in the BAM confirmed the absence of cells (Fig. 1G–J). The data demonstrated that the decellularization process removed all the residual cells from the bladder and retained its natural structure.

The conjugation efficiency of Traut's and Sulfo-SMCC

As indicated in Figure 2A, the amount of sulfhydryl groups increased in a concentration-dependent manner from 0 to 5 mg/mL, and the sulfhydryl groups became saturated above 5 mg/mL. Therefore, 5 mg/mL Traut's reagent was chosen for subsequent studies. To detect the Sulfo-SMCC conjugation efficiency, the BAM scaffold treated by 5 mg/mL Traut's reagent was reacted with bFGF treated with different molar excess of SMCC. The SMCC concentration-dependent curve reached the peak at the molar excess of 60-fold to bFGF, and 60 molar excess of SMCC was used for the following experiments (Fig. 2B).

Ultrastructure change after conjugation

Both the BAM and CL-BAM scaffold retained its three-dimensional space structure with highly interconnected pores, which were suitable for cell infiltration and ingrowth. The morphology of BAM was loose (Fig. 3A), while CL-BAM scaffolds were expanded and compressed (Fig. 3B) compared with BAM under freeze-dried conditions.

Binding and release ability

As shown in Figure 4A, the results of ELISA indicated that the amount of bFGF bound on both BAM and CL-BAM increased in a concentration-dependent fashion, and CL-BAM could retain more bFGF than BAM. The differences between CL-BAM and BAM at the concentration of 1, 2, 4, and 8 μ M were significant. In the releasing buffer, the BAM group with simple adsorption displayed the typical burst release during the first 24 h, whereas the CL-BAM group released bFGF in a controlled manner within 96 h (Fig. 4B).

The CL-BAM/bFGF system promoted the proliferation of fibroblasts in vitro

Human fibroblasts grew well on both the BAM/bFGF scaffold and the CL-BAM/bFGF scaffold, but the CL-BAM/bFGF scaffold had higher cell density (Fig. 5A–C). Human fibroblasts in both groups showed a time-dependent proliferation. There was a statistically significant difference of cell proliferation at 3, 5, and 7 days ($p < 0.01$) (Fig. 5D).

FIG. 4. Binding and release of basic fibroblast growth factor (bFGF) from BAM after crosslinking treatment. (A) bFGF binding curves of CL-BAM group and BAM group measured by ELISA assay. (B) Release of bFGF from CL-BAM group and BAM group measured by ELISA assay. Data are presented as mean $\pm$ SD. * $p < 0.05$ and ** $p < 0.01$.

FIG. 5. Biological activity of bFGF conjugated onto BAM scaffold by stimulation of fibroblast proliferation. (A) Phase-contrast image of BAM scaffolds for cell culture. (B) Fibroblasts stained by fluorescein diacetate (FDA) on BAM/bFGF scaffolds. (C) Fibroblasts stained by FDA on CL-BAM/bFGF scaffolds. (D) Proliferation of fibroblasts was measured by the MTT method. Data are presented as mean $\pm$ SD. * $p < 0.05$ and ** $p < 0.01$. Color images available online at www.liebertpub.com/tea

The CL-BAM/bFGF system promoted cellularization and vascularization after subcutaneous implantation

As shown in Figure 6A–L, after subcutaneous implantation for 2 weeks, macro-observation showed no visible inflammations or signs of tumor around the samples. The amount of cells were 305.67 ± 31.08 in BAM, 551.83 ± 45.15 in BAM/bFGF, 618.17 ± 80.79 in CL-BAM, and 1046.5 ± 87.04 in CL-BAM/bFGF. The number of blood vessels per field ($n = 6$) was 7 ± 2 in BAM, 14.67 ± 2.16 in BAM/bFGF, 14.66 ± 3.38 in CL-BAM, and 29.83 ± 3.82 in CL-BAM/bFGF. The statistical analysis indicated that there was a significant difference among these groups as shown in Figure 6M, N.

The CL-BAM/bFGF system promoted bladder regeneration

All the rats survived before their scheduled sampling time. There were no evidences about urinary leaks or extravasations of urine into the abdominal cavity. Bladder stones were found in four of the BAM group, two of the CL-BAM group, and one of the BAM/bFGF group, which were not found in the CL-BAM/bFGF group. As shown in Figure 7, the CL-BAM/bFGF group revealed better tissue regeneration merged into the normal surrounding bladder tissues and less shrink. H&E staining indicated that urothelial layers and smooth muscle bundles could grow into scaffolds. The percentage of α -SMA-positive area in the CL-BAM/bFGF group (35.83 ± 3.43) was significantly higher compared with the BAM group (6.17 ± 2.14), BAM/bFGF group (13.83 ± 3.19), and CL-BAM group (14.17 ± 3.31) ($p < 0.01$, respectively), still lower than that in the sham-operated group (47.50 ± 4.14 , $p < 0.05$). The number of blood vessels in the CL-BAM/bFGF group (11.67 ± 1.37) was significantly higher compared with the BAM group (4.67 ± 0.82), BAM/bFGF group (7.67 ± 0.82), and CL-BAM group (7.05 ± 1.05) ($p < 0.01$, respectively), still lower than that in the sham-

operated group (18.83 ± 4.71 , $p < 0.01$). As shown in Figure 8, the implants of the CL-BAM/bFGF group were stronger compared with other scaffold groups with statistical differences. The mean values of ultimate tensile strength in each group were as follows: BAM: 1.34 ± 0.3 N; BAM/bFGF: 2.46 ± 0.24 N; CL-BAM: 2.34 ± 0.33 ; CL-BAM/bFGF: 2.98 ± 0.35 N; and sham operated: 4.16 ± 0.26 N. There were no statistical differences in the bladder volume capacity and maximum intravesical pressure among the four groups, but the bladder compliance, a measurement of the elastic properties opposing a change in volume per unit of change in pressure, was significantly higher in the CL-BAM/bFGF than that of the other scaffold groups (Table 1). There was no statistically significant difference in compliance between the CL-BAM/bFGF group and sham-operated group ($p > 0.05$).

Discussion

Restricting growth factors in scaffolds can maintain therapeutic concentrations and decrease the side effects. Recently, a number of studies have investigated the delivery vehicles for growth factor release. Based on gene fusion, growth factors have been developed as fusion proteins by recombinant expression from gene sequence encoding target-binding domains, which can bind the substrate specifically.¹⁷ Based on chemical crosslinking, heparin has been conjugated onto biomaterials to retain growth factors.^{18,19} Encapsulation of growth factors in hydrogel has been demonstrated as sustained release and an excellent candidate for cytokine delivery.^{20,21} Compared with other bFGF controlled release methods, the importance and advantage of crosslinking bFGF on BAM is that it does not need some special substrates or binding domains. By chemical crosslinking, the biostability and mechanical strength of the scaffold can be increased, and it promotes cell migration, viability, and differentiation.²²

In this study, BAM was selected to deliver bFGF because its native architectural structure was favorable for bladder cells to

FIG. 6. Histological evaluation for cellularization and vascularization of the scaffolds 2 weeks after subcutaneous implantation. BAM group (A, E, I), BAM/bFGF group (B, F, J), CL-BAM group (C, G, K), and CL-bFGF/bFGF group (D, H, L). Gross analysis (A–D). H&E staining (E–H). Immunohistochemistry examination with anti-von Willebrand factor antibody (vWF) (I–L). Scale bar = 100 μ m. (M) The statistical analysis of cell numbers growing into BAM scaffolds per field. (N) The statistical analysis of vessel numbers growing into BAM scaffolds per field. Data are presented as mean $\pm$ SD. * p < 0.05 and ** p < 0.01. Color images available online at www.liebertpub.com/tea

recognize and grow in. Since collagen-based BAM contained many primary amines and carboxyl groups, it could be modified for chemical conjugation using Traut's reagent and SMCC.²³ This reagent has been used to conjugate antibodies or enzyme proteins without affecting their biological activity.^{24,25} Recently, this reagent has been also used to immobilize relevant molecules to biomaterials.^{23,26}

After conjugation, BAM maintained a higher concentration and could achieve controlled release of bFGF. In a rat model, the

CL-BAM/bFGF group was observed to produce more desirable results of bladder tissue regeneration. The conjugation of bFGF to the BAM scaffolds maintained bFGF at the target site, which prevented the diffusion of bFGF by body fluids, and thus could promote more effective tissue regeneration.

There was not much difference of the repair effects between the BAM/bFGF and CL-BAM group. We speculated that the chemical treatment might improve the physical property of the scaffolds, which could maintain the pore sizes and avoid

FIG. 7. Evaluation of the tissue regeneration effect of the scaffolds at 90 days after operation. BAM group (A, F, K, P), BAM/bFGF group (B, G, L, Q), CL-BAM group (C, H, M, R), CL-BAM/bFGF group (D, I, N, S), and sham-operated group (E, J, O, T). Gross analysis (A–E). H&E staining (F–J). Immunohistochemical staining for α -smooth muscle actin (α -SMA) (K–O). Immunohistochemistry examination with anti-vWF (P–T). Scale bar = 100 μ m. (U) The statistical analysis of the percentage of α -SMA-positive area. (V) The statistical analysis of the capillary numbers counted from six randomly selected fields. * $p < 0.05$ and ** $p < 0.01$, determined by one-way ANOVA. Color images available online at www.liebertpub.com/tea

shrinking after implantation and it would be more conducive to cell anchorage.²² Natural collagen breaks down so fast that it is not suitable as a scaffold for long-term tissue regeneration. Some studies showed that crosslinking could delay the degradation rate of the scaffold.^{22,23} The lower degradation rate *in vivo* could enhance the anchorage and ingrowth of endogenous cells and

avoid shrinking and crimpling by fibrous tissue around the scaffolds. When Sulfo-SMCC and Traut's reagent was used for conjugating bFGF on BAM, they also promoted the inner self-crosslinking of BAM, which might delay the degradation time of BAM after being implanted into a rat bladder. In addition, a large number of free sulfhydryl groups onto the BAM scaffolds were

FIG. 8. Mechanical properties of implanted biomaterials at 90 days after surgery ($n=6$). (A) Schematics of mechanical tests; (B) ultimate tensile strength of each group; (C) the statistical analysis of the ultimate tensile strength. Data are presented as mean $\pm$ SD. * $p < 0.05$ and ** $p < 0.01$. Color images available online at www.liebertpub.com/tea

introduced by Traut's reagent. It was reported that free sulfhydryl groups could activate various biological responses, including platelet activation,²⁷ which might accelerate the cellularization and vascularization of the scaffolds.

Although the CL-BAM/bFGF target delivery system shows some promising results in tissue regeneration, many issues still remain to be resolved in the future studies. First, chemical treatment might change the physical properties of the BAM as indicated in Figure 3. Therefore, it is important for the conjugated scaffold to balance the rate between controllable degradation and tissue replacement. Second,

the limited graft size in rodent models provides an inadequate evidence of bladder regeneration due to the rate at which the regenerative process occurs. Furthermore, one growth factor may be not enough to meet the needs for successful tissue regeneration. A combination of cell-seeded scaffold-conjugated multiple growth factors needs to be evaluated in large animal models before clinical applications.

Conclusion

This study demonstrated that BAM conjugated by Traut's reagent and SMCC could bind with bFGF specifically and achieve controlled release of bFGF. This system retained its biological activity and showed therapeutic potential for bladder regeneration in a rat model. The results suggest that chemically modified scaffolds can be used as a potential alternative approach for growth factor delivery and tissue regeneration.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (31000442, 81230017).

TABLE 1. URODYNAMIC STUDY 90 DAYS AFTER SURGERY

	Capacity (mL)	Maximum pressure (cmH ₂ O)	Compliance (cmH ₂ O)
BAM	2.235 $\pm$ 0.188	40.037 $\pm$ 5.332	0.157 $\pm$ 0.016
BAM/bFGF	2.339 $\pm$ 0.196	41.236 $\pm$ 4.775	0.198 $\pm$ 0.025 ^a
CL-BAM	2.401 $\pm$ 0.231	40.979 $\pm$ 5.941	0.208 $\pm$ 0.0218 ^a
CL-BAM/bFGF	2.411 $\pm$ 0.176	42.658 $\pm$ 4.591	0.233 $\pm$ 0.0304 ^b
Sham operated	2.399 $\pm$ 0.219	43.719 $\pm$ 3.887	0.240 $\pm$ 0.0298 ^b

Data are presented as mean $\pm$ SEM.

^a $p < 0.05$ and ^b $p < 0.01$.

Disclosure Statement

No competing financial interests exist.

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Received: December 1, 2013

Accepted: January 29, 2014

Online Publication Date: July 3, 2014