Bioactive Porous Beads as an Injectable Urethral Bulking Agent: In Vivo Animal Study for the Treatment of Urinary Incontinence

In Gul Kim, M.S.,1,2 Se Heang Oh, Ph.D.,1 Ji Young Lee, M.S.,2 Ji Youl Lee, M.D., Ph.D.,2 and Jin Ho Lee, Ph.D.1

In our previous study, growth factor (basic fibroblast growth factor [bFGF] or vascular endothelial growth factor)-immobilized polycaprolactone (PCL)/Pluronic F127 porous beads were fabricated by an isolated particle-melting/melt-molding particulate-leaching method. The growth factors were easily immobilized onto the pore surfaces of the PCL/F127 beads via heparin binding, and were continuously released for up to 28 days. In this study, the growth factor-immobilized porous beads were investigated for their potential use as an injectable urethral bulking agent for the treatment of stress urinary incontinence (SUI). From the in vivo study using Sprague-Dawley rats as an urinary incontinent animal model, it was observed that the growth factor (bFGF or vascular endothelial growth factor)-immobilized porous beads had effective cure behaviors for SUI as follows: the narrowed urethral lumen and the regeneration of smooth muscle around the urethra. In particular, the bFGF-immobilized PCL/F127 porous beads showed desirable smooth muscle regeneration and electrical contractility, which indicates it can be a good candidate as an injectable bioactive bulking agent for the treatment of SUI.

Introduction

Stress urinary incontinence (SUI), which is defined as the involuntary leakage of urine upon physical activity, sneezing, or coughing, is considered a common and embarrassing problem among women.1 SUI occurs when the ordinary abdominal pressure exceeds the urethral closing pressure, and it is understood that this symptom is associated with a lack of urethral support caused by damage and/or weakness of tissues (urethral sphincter, pelvic floor muscle, and nerve) around the urethra.2 The key factors in the development of SUI have been referred to the aging, which may reduce the number of muscle fibers and nerve density around the urethra,3–5 and childbirth, which can lead to damage of the urethral sphincter, pelvic floor muscle, and nervous structure during vaginal delivery.5–8 All treatments for SUI are based on the incremental strengthening of urethral resistance during periods of increased abdominal pressure. They include pharmacologic therapy, surgical repair, and injection of a bulking agent.9,10 The pharmacologic therapy can be applied for only relatively mild SUI symptoms and cannot effectively improve the urethral resistance to prevent the loss of urine in the case of serious tissue damage around the urethra.9 The bulking agent has usually been used as a first-line therapy in the treatment of SUI because of its initial low cost, minimal invasiveness, and low morbidity as compared to surgical repair methods, such as colposuspension11 or the sling technique.12,13 It has been suggested that the narrowing of the urethral lumen due to the injected obstructive component under urethral mucosa is the SUI cure mechanism via bulking agent injection.9,10 A variety of injectable biomaterials that can provide the appropriate volume at the applied site, such as glutaraldehyde-crosslinked bovine collagen (Contigen®), autologous fat, silicone-based particle (Macroplastique®), carbon-coated zirconium particle (Durasphere®), and dextranomer/hyaluronic acid copolymer (Zuidex™), are currently used for the treatment of SUI.10 However, biologic reabsorption, particle migration, and ongoing degradation of the injected bulking agents, and thus the need to perform multiple injections still remain as major clinical problems. This suggests that the cure rate of conventionally used bulking agents relies on only the passive bulking effect due to the volume injected. In recent studies, many researchers believed that the regenerative therapy based on cells and growth factors can be an attractive therapeutic technique for the treatment of SUI.14,15 It is well known that the cells and growth factors can allow for the replacement, repair, and functional recovery of defected tissues around the urethra. Several research groups have demonstrated that stem cells or progenitor cells injected into
the urethral wall can improve sphincter function via the regeneration of muscles and nerves in animal models and in clinical applications. It was also reported that the continuous release of growth factor from a hydrogel can regenerate the urethral muscles and thus improve the sphincteric contractility.

In our previous in vitro study, we demonstrated that growth factor [basic fibroblast growth factor (bFGF) or vascular endothelial growth factor (VEGF)]-immobilized hydrophilic polycaprolactone (PCL) porous beads can promote smooth muscle cell (SMC) differentiation of muscle-derived stem cells (which are found in the urethral sphincter and are believed to be involved in sphincter regeneration) by the sustained release of the growth factor. In this study, we expected that effectively growth factor-releasing hydrophilized porous beads may be a promising bioactive bulking agent for the stimulation of the regeneration of smooth muscles around the urethra (due to the growth factor release) as well as to provide a bulking effect (due to the injected porous bead volume), and thus achieve the ultimate SUI treatment. To confirm this, the growth factor (bFGF or VEGF)-immobilized PCL/Pluronic F127 porous beads were injected into the urethral wall of an urinary incontinent rat model (refer to Fig. 1), and their effects on the leak point pressure (LPP) (passive bulking effect), smooth muscle regeneration, or angiogenesis around the urethra and the functional recovery of urethra tissues (electrical contractility) (bioactive effects) were investigated. Both bFGF and VEGF are well known as growth factors that induce smooth muscle differentiation as well as angiogenesis. To our knowledge, no studies using bulking agents for the treatment of SUI have been carried out for the biological and functional recovery of the urethral sphincter as well as for the passive bulking effect.

**Materials and Methods**

**Materials**

PCL (MW 43,000–50,000; Polyscience) and Pluronic F127 (MW 12,500; Sigma) were used to fabricate hydrophilic porous beads. Heparin and growth factors (bFGF and VEGF) were purchased from Celsus Laboratories and R&D Systems, respectively. All other chemicals were of analytical grade and used as received. Water was purified using a Milli-Q purification system (Millipore Co.). For the animal study, the PCL/Pluronic F127 porous beads were sterilized by ethylene oxide and a Pluronic F127 solution used as a carrier for the uniform injection of porous beads was autoclaved.

**Preparation of growth factor-immobilized porous beads**

PCL/Pluronic F127 porous beads were fabricated by the modification of an isolated particle-melting method (for nonporous beads) and a subsequent melt-molding particulate-leaching method (for porous beads) described elsewhere. Briefly, PCL pellets were crushed into micro-sized particles (random-shape) and sieved with a size range of 100–150 μm, and then the crushed particles were dispersed in a cold Pluronic F127 aqueous solution [F127 solution, 20 wt%; PCL particles/F127 solution ratio, 1/50 (w/v)]. The PCL particles-dispersed Pluronic F127 solution was kept at room temperature for 1 h to induce the gelation of Pluronic F127 (sol–gel transition temperature of the Pluronic F127 solution, ~20°C). Then, the particles evenly dispersed in the

---

**FIG. 1.** Schematic diagrams showing the preparation of the bioactive bulking agent, its injection into the urethral wall, and the possible cure mechanism for the urinary incontinence. Color images available online at www.liebertonline.com/tea
gel matrix were melted in a water bath (65°C) for 30 min. During this step, the random-shape PCL particles were melted (melting point of PCL, ~60°C) and transformed to spherical shapes individually in the gel matrix without the fusion among the particles (each particle is isolated in the gel matrix like an island). After this treatment, the PCL beads (spherical, nonporous)/Pluronic F127 gel mixture was cooled down to ~4°C and centrifuged to obtain the Pluronic F127-coated PCL nonporous beads. To fabricate PCL/Pluronic F127 porous beads, the Pluronic F127-coated PCL nonporous beads, and sodium chloride particle (NaCl; size range of 25–50 μm) mixtures (1/40 v/w ratio; 2.5 g) were placed in a brass mold (diameter, 18 mm; height, 2.5 mm) and the mold was thermally compressed at 80°C using a compression molding press. The salt particle-embedded PCL/F127 beads were immersed in excess water to leach out salts and free Pluronic F127 from the beads. The PCL/Pluronic F127 beads were immersed in excess water to leach out salts and free Pluronic F127 from the beads. The PCL/Pluronic F127 porous beads (size range of 200–300 μm) were obtained after vacuum drying overnight. Their surface and cross-sectional morphologies were observed by a scanning electron microscope (Model S-3000N; Hitachi).

Two different growth factors, bFGF and VEGF, were incorporated onto the pore surfaces of the PCL/F127 beads via heparin immobilization. To do this, the prepared porous beads were soaked in the heparin solution (1 mg/mL [in 2 wt% NaCl solution]) at 4°C for 3 h. After thorough washing with 2 wt% NaCl solution and water, the heparin-immobilized PCL/F127 porous beads were soaked in two different growth factor solutions (bFGF and VEGF, each 200 ng/mL) at room temperature for 3 h. The growth factor-immobilized porous beads were obtained after washing with phosphate-buffered saline (pH 7.4) and vacuum drying overnight.

**Implantation of growth factor-immobilized porous beads**

Sprague-Dawley rats, weighting 200–250 g, were chosen as an urinary incontinent animal model to evaluate the passive bulking and bioactive effects of the growth factor-immobilized PCL/F127 porous beads injected into the urethral wall. All animal experiments were approved by the Animal Care Committee of the Catholic University of Korea, and all procedures were performed according to the appropriate guidelines. Surgery was performed under general anesthesia. The anesthesia was induced by an intramuscular injection of tiletamine/zolazepam (10 mg/kg; Zoletil 50®, Virbac Laboratories) and 2% xylazine hydrochloride (2 mg/kg; Rumpun®, Byely). The animals, a total of 30, were divided into five groups (each group, six animals): a normal group without urinary incontinence (normal), a urinary incontinent group (urinary incontinence), a PCL/F127 beads-injected urinary incontinent group (beads w/o GF), and VEGF- and bFGF-immobilized PCL/F127 bead-injected urinary incontinent groups (VEGF beads and bFGF beads). The urinary incontinent rats (all groups except the normal group) were prepared by the denervation of their sciatic nerve. The sciatic nerve on each side was exposed through a bilateral dorsal incision and transected distal to its origin from the sciatic nerve trunk. At 1 week after nerve transection, the urinary incontinent rats were placed in a dorsal position and a low midline incision was made to expose the bladder and urethra. Then, the porous beads (with or without growth factors) were carefully injected into the periurethra submucosa with microscopic guidance to minimize tissue damage. Each injection (20 μL/rat) was given through a syringe with a 19G needle. For the even delivery of the porous beads by injection, the beads were uniformly dispersed in a cold Pluronic F127 aqueous solution (25 wt%; sol-gel transition temperature, about 17°C) with a beads to solution ratio of 1/12 (w/v). The porous beads formed a well-packed structure in the solution, which can allow stable volume retention even after the clearance of Pluronic F127 in the body. It was reported that microparticles having sizes above 80 μm for bulking agent application can prevent effectively from their migration. The beads in the gel state of the Pluronic F127 at room temperature could be homogeneously injected through the syringe needle without the separation of the beads and Pluronic F127 gel. Figure 1 shows the overall scheme of the preparation of the bioactive bulking agent (growth factor-immobilized porous beads dispersed in Pluronic F127 gel), its injection into the urethral wall, and the possible cure mechanism for the urinary incontinence.

**LPP measurement**

At 4 weeks after the periurethral injection of the porous beads (with or without the growth factors), the LPP to evaluate the physical urethral sphincteric function was measured using a vertical tilt/intravesical pressure clamp model. Before the LPP testing, the spinal cord was transected at the T9 level to avoid spontaneous bladder responses by the increasing intravesical pressures, whereas the rats were under anesthesia of rats. This treatment does not interfere with the spinal continence reflexes of the bladder neck and urethra. The feces in the distal colon and rectum were evacuated by gentle massage via a midline abdominal incision, and a loose suture was secured around the proximal end of the distal colon to prevent any further migration of feces which can affect the LPP. Thereafter, a PE-90 transvesical catheter was inserted into the dome of the bladder and secured with a ligature for bladder filling. The muscle and skin incision was closed with sutures. The rat was then mounted on a tilt table and placed in a vertical position. Intravesical pressure was controlled by the height adjustment of a saline reservoir attached on a metered vertical pole. The reservoir outlet was connected with the transvesical catheter via a 2-way stopcock. The intravesical pressure was increased in 1–3 cm H2O steps until the visual identification of leakage, and the leak point was determined as the LPP. To guarantee the reliability of the results, all measurements were processed in triplicate.

**Histological and immunohistochemical analyses**

The animals that underwent the LPP measurement were immediately sacrificed by an overdose of CO2 gas, and the proximal urethra, which included the injected porous beads, was carefully dissected. After being frozen at ~20°C, the specimens were vertically cut into 4 μm thicknesses to investigate the smooth muscle or blood vessel regeneration by the sustained release of the growth factors (bFGF or VEGF) from the porous beads (bioactive effect). The sections were mounted on positively charged slides and stained with hematoxyline and eosin (H&E) for observation by light
microscopy (Olympus). The urethral lumen dimensional change due to the injected porous beads (passive bulking effect) was also investigated, by measuring the area of urethral lumen using an image analysis program (i-solution, IMT) from the H&E staining images.

Immunohistochemical staining was also conducted for anti-alpha smooth muscle actin (anti-αSMA) and anti–von Willebrand factor (anti-vWF) antibodies. To evaluate the smooth muscle or blood vessel regeneration behavior around the porous beads (with or without growth factors) injected into the urethra, the slides were washed with PBTx (0.1% Triton X-100 in phosphate-buffered saline), blocked with 1% bovine serum albumin (Amresco), and 1.5% normal goat serum (Vector laboratories) in PBTx at 37°C for 1 h. To observe the regenerated smooth muscle and blood vessels, the sections were incubated at 4°C overnight with anti-αSMA antibody (diluted to 1:200; Abcam) and anti-vWF antibody (diluted to 1:50; Chemicon), respectively. They were also incubated with secondary antibodies [Alexa Fluor® 488 goat anti-rabbit IgG; Invitrogen (for anti-αSMA antibody) or Alexa Fluor® 568 goat anti-rabbit IgG; Invitrogen (for anti-vWF antibody)] in 1% bovine serum albumin and 1.5% normal goat serum in PBTx at room temperature for 1 h. Then, a coverslip was mounted on the slide using a mounting medium with 4¢,6-diamidino-2-phenylindole (Vector laboratories) to observe the cell nuclei. The slide was observed by a fluorescent microscope (Model BX51; Olympus).

**Contractility test**

For the contractility test, the animals were sacrificed at 4 weeks after the periurethral injection of the porous beads (with or without growth factors), and the proximal urethra, which included the injected porous beads, was dissected, trimmed, weighed, and detubularized in a spiral fashion to produce a 1×10 mm² tissue strip. One end of the strip was connected with a 3–0 silk suture to a glass hook, and the other end was tied to an isometric force transducer (FT03; Grass Instruments). The strip was suspended in a 20 mL organ bath containing Tyrode's solution (116 mM NaCl, 5 mM KCl, 5 mM HEPES, 1 mM MgCl₂, 24 mM NaHCO₃, 2 mM CaCl₂, and 11.5 mM glucose) bubbled with a mixture of 95% O₂ and 5% CO₂ at 37°C. Before the electrical field stimulation, the strip was equilibrated at 1 g initial tension for 30 min in the organ bath. Then, the strip was stimulated at 32 Hz with 1 ms pulses at 80 volts using an S48 stimulator (Grass Instruments) for 30 s. Contractions of the strip caused by the electrical field stimulation were measured through the isometric force transducer and were recorded on a personal computer by the use of a commercial data acquisition system (PowerLab®; AD Instruments). A contractile response, which is expressed by tension per unit weight of strip, was also obtained from the contraction results.

**Statistical analysis**

The data obtained from each group were averaged and expressed as mean ± standard deviation. The Student's t-test was used to determine the significance of the differences between the groups. The differences were considered statistically significant at \( p < 0.05 \).

**Results**

**Characterization of growth factor-immobilized porous beads**

The PCL/F127 porous beads fabricated by modified isolated particle-melting (for nonporous beads) and subsequent melt-molding particulate-leaching (for porous beads) methods exhibited highly porous surface and interior pore structures, which can provide a large surface area for growth factor immobilization (bead size range, 200–300 μm; Fig. 2). The pore sizes in the beads were almost the same as the salt particle sizes used (salt particle size range of 25–50 μm).

**LPP evaluation**

Figure 3 shows that the LPP results at 4 weeks after injection of the porous beads. The porous beads/Pluronic F127 gel mixture was easily injected through a 19G needle until the syringe was empty because of the highly viscous Pluronic F127 gel acting as a bead carrier and a lubricant for the injection system. It was observed that the mean LPP of the urinary incontinent group (24.1±2.4 cm H₂O) was significantly lower than the normal group (36.8±1.0 cm H₂O), indicating that the urinary incontinence of the rats was...
effectively induced by the denervation of the sciatic nerve. The mean LPPs of all porous bead groups were not significantly different from that of the normal group (beads w/o GF, 33.3 – 6.5; VEGF beads, 38.5 – 2.2; and bFGF beads, 37.5 – 7.1 cm H2O), indicating that the injected porous beads can provide an appropriate passive bulking effect around the urethra.

**Histological and immunohistochemical evaluations**

The animals that underwent the LPP measurement were sacrificed to obtain their proximal urethra tissue, which included the injected porous beads. At explantation, any inflammatory signs or adverse tissue reactions were not observed. The H&E staining results at 4 weeks after injection of the porous beads are shown in Figure 4. The urethral submucosa of the normal group had a longitudinally oriented thick smooth muscle layer and a circular striated muscle layer, which can provide ordinary urethral sphincteric function to prevent the involuntary loss of urine (Fig. 4A). On the other hand, the smooth muscle layer of the urinary incontinent group was significantly reduced (muscle atrophy due to the denervation), and thus the urethral lumen diameter was notably larger compared to that of the normal group, which can lead to diminished urethral closing pressure (Fig. 4B). For the porous bead-injected groups (Fig. 4C–E), it was observed that the beads were correctly injected around the urethral submucosa of the urinary incontinent rat and stably maintained at the applied site without particle migration. The Pluronic F127 gel used as a carrier for the homogeneous injection of the porous beads did not lead to any adverse tissue responses, probably because of its fast absorption in the body (within a few days) as well as its own biocompatibility. The PCL as well as Pluronic F127 are well known as being biocompatible and were approved by FDA, for human use. The urethral lumens of each porous bead group (regardless of the presence of growth factor) were comparably narrowed with the normal group due to the injected bead volume, indicating an effective bulking effect (Area of urethral lumen: normal, 0.14 ± 0.02 mm²; urinary incontinence, 0.31 ± 0.07 mm²; beads w/o GF, 0.17 ± 0.03 mm²; VEGF bead, 0.14 ± 0.04 mm²; bFGF bead, 0.16 ± 0.05 mm²). This observation was consistent with the result of the LPP measurement that the porous bead groups showed similar LPP values as the normal group, while the urinary incontinent group had a significantly lower LPP than the normal group (refer to Fig. 3).

The immunohistochemical analyses using anti-αSMA and anti-vWF antibodies at 4 weeks after the porous bead injection were conducted to estimate whether the bFGF or VEGF immobilized on the porous beads can induce smooth muscle or blood vessel regeneration around the urethra, and thus...
improve the sphincter function (Figs. 5 and 6). The smooth muscle can be stained positively by an anti-αSMA antibody and it can be expressed in green color in a fluorescence image. It was observed that the normal group showed a strong green color within the urethral submucosa (Fig. 5A), which indicates thick bundles of tightly packed smooth muscle and thus can allow for the controlled storage and timely disposal of urine. On the other hand, the urinary incontinent group showed an extensively disrupted and thinning smooth muscle layer (weakened green color), caused by the denervation (Fig. 5B). The injection of the porous beads without growth factor into the perirectal area reduced the urethral lumen diameter due to the injected volume, but any smooth muscle regeneration around the urethra could not be found (Fig. 5C), indicating that the porous beads caused the passive bulking effect at the applied site. However, the growth factor (VEGF or bFGF)-immobilized porous beads additionally allowed for the smooth muscle regeneration around the urethra (bright green color) (Fig. 5D, E). The bFGF-immobilized bead group showed a little denser smooth muscle regeneration than that of the VEGF-immobilized group. Blood vessel formation was also observed around the VEGF and bFGF-immobilized porous beads, particularly VEGF-immobilized beads (Fig. 6D), which may provide an ideal environment for the survival and maturation of the regenerated smooth muscle.

**Contractility**

To evaluate the biological sphincteric functional recovery of the urethral tissue by smooth muscle or blood vessel regeneration, the electrical contractility test was conducted at 4 weeks after injection of the porous beads. Figure 7 shows the representative contractility recording of the urethral strips at 4 weeks after injection of the porous beads. The normal urethral strip strongly contracted in response to the electrical stimulations, which implies sound sphincteric function (Fig.
but the urethral strip of the urinary incontinent group (Fig. 7B) and the group of porous beads without the growth factor (Fig. 7C) showed very weak contractions (similar to noise due to the stimulus), probably because of atrophy of the smooth muscle around the urethra (refer to Fig. 5). On the other hand, the urethral strips of the VEGF and bFGF-immobilized porous bead groups (Fig. 7D, E) showed greater contractility compared to the groups of urinary incontinence and beads without growth factor. In particular, the bFGF-immobilized bead group exhibited more robust contractions than the VEGF group. The contractile response expressed by tension per unit weight of each urethral strip is shown in Figure 8. The contractile response of the normal, urinary incontinence, beads w/o GF, VEGF bead, and bFGF bead groups were 35.5–2.4, 7.7–1.3, 11.7±1.8, 21.5±1.6, and 27.5±2.3 g tension/mg tissue, respectively. The bFGF-immobilized bead group had a significantly improved contractile response compared to the other groups except for the normal group.

Discussion

It is well understood that SUI is directly correlated to the functional disorders of the bladder, sphincter, and pelvic organ support and neuronal commands caused by aging and childbirth, and the symptom can be sufficiently relieved by bulking agent injection, which can produce a local tissue elevation due to the injected volume and hence lead to urethral closure, as discussed earlier. From this point of view, the combination of functional recovery of the damaged tissues (bioactive property) and bulking effect (passive property) may be a fundamental therapeutic technique for SUI. In this study, the growth factor (bFGF or VEGF)-immobilized PCL/Pluronic F127 porous beads, which can be easily applied at the periurethra by noninvasive injection, were fabricated to investigate their potential use as a bioactive bulking agent for SUI. We expected that the growth factor-immobilized hydrophilized porous beads (PCL/F127 beads) may provide a bulking effect at the injected site (due to the porous beads) and stimulate the regeneration of defected tissues around the urethra (due to the sustained release of the growth factors).

The prepared PCL/F127 porous bead sizes were ranged from 200 to 300 μm with pore size ranges of 25–50 μm (Fig.

FIG. 7. Representative electrical contraction recording of the urethral strips at 4 weeks after the injection of the porous beads. (A) normal, (B) urinary incontinence, (C) beads w/o GF, (D) VEGF bead, and (E) bFGF bead groups. Color images available online at www.liebertonline.com/tea

FIG. 8. Contractile response of the urethral strips at 4 weeks after the injection of the porous beads (with or without growth factors) (n=3; *p<0.05).
After injection into the body, it was expected that the porous beads can be stably located at the applied site without particle migration because of their sizes. It was reported that the required particle sizes to prevent migration in the body are above 80 μm for bulking agent application.10 Moreover, the surrounding tissues gradually infiltrated into the porous bead region can also provide long-term volume retention.26 In our previous study,25 we reported that bFGF and VEGF were effectively immobilized onto the porous surface of the PCL/Pluronic F127 beads through heparin binding and were released in a sustained manner for up to 28 days. The growth factors, being continuously released from the hydrophilized PCL/F127 porous beads, effectively induced SMC differentiation of the muscle-derived stem cells, suggesting that the growth factors may effectively stimulate the regeneration of smooth muscles around the urethra for improved sphincter function, as discussed earlier.

To estimate the functional and histological repair of urethral sphincter of urinary incontinent rats due to the injected growth factor-immobilized porous beads, the LPP and H&E staining were conducted at 4 weeks after their injection. The significantly lower LPP (Fig. 3) and the notably larger urethral lumen diameter (Fig. 4B) of the urinary incontinent group compared with those of the normal group indicate that the urinary incontinence of the rats was effectively induced by the denervation of the sciatic nerve. It was reported that direct injury to the sphincter muscle around the urethra and the injury to the sciatic nerve with subsequent denervation to the rat sphincter can result in urinary incontinence.28 We also observed that the LPPs of all porous bead groups were not significantly different from that of the normal group, and the urethral lumens of each porous bead group (regardless of the presence of growth factor) were comparably narrowed with the normal group due to the injected bead volume. This suggests that the injected porous beads (regardless of the presence of growth factors) provided an appropriate bulking effect around the urethra and thus prevented the leakage of urine for the treatment of urinary incontinence. It was well recognized that the SUI cure mechanism by the bulking agent injection is deeply influenced on the narrowing of the urethral lumen due to the injected volume under urethral mucosa, as discussed earlier.9,10

The growth factor (VEGF or bFGF)-immobilized porous beads allowed for the smooth muscle regeneration around the urethra (Fig. 5D, E). Particularly, the bFGF-immobilized bead group was effective for the smooth muscle regeneration and significantly improved contractile response (functional recovery) compared with the other urinary incontinent groups (Figs. 7 and 8). The smooth muscle regeneration around the urethra and its functional recovery may be explained by the proliferation of SMCs,30–32 and/or the SMC differentiation of the stem cells or progenitor cells found around the urethra25 by the sustained release of bFGF from the porous beads. The regenerated smooth muscle around the urethra was expected to be very helpful for biological sphincteric functional recovery.

In conclusion, the bFGF-immobilized PCL/Pluronic F127 porous beads seem to provide a more appropriate environment for smooth muscle regeneration in our system. The exact mechanism that explains how the bFGF released from the porous beads leads to better smooth muscle regeneration and electrical contractility than VEGF is not yet clear. However, we can speculate that the bFGF-immobilized porous beads can be a promising bioactive bulking agent system that can improve the sphincter muscle function around the urethra (due to the regenerated smooth muscle) as well as provide the passive bulking effect (due to the porous beads), and thus can fundamentally treat SUI. Moreover, we recognized that if the growth factor-immobilized porous beads, which can provide an appropriate environment for cell adhesion and proliferation (by high porosity and interconnectivity) as well as differentiation into target cells (by sustained release of growth factors), are combined with the emerging stem cell-based therapy, this may become an advanced therapeutic technique for the treatment of SUI.

Acknowledgments

This research was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2010–0002176).

Disclosure Statement

No competing financial interests exist.

References


