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Development of Bilayered Bone Marrow-derived Cell-Gelatin Grafts for Augmentation Cystoplasty and Reconstruction of Bladder Tissues in Rats

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Background : This study attempted to produce a novel graft composed of bone marrow-derived mesenchymal cell (BMC) layer-gelatin sheets for bladder augmentation cystoplasty. Then, we determined if the grafts could reconstruct bladder tissues.

Methods : BMCs harvested from the femurs of green fluorescence protein (GFP)-transfected Sprague-Dawley (SD) rats were adherent and proliferating cells on collagen dishes. The cells were then cultured on temperature-responsive culture dishes. Following this, the BMCs maintaining cell-cell contacts within the monolayer itself were applied to a gelatin sheet. Two BMC layer-gelatin sheets were overlaid together with the cell sides juxtaposed with one another (bilayered BMC-gelatin graft). Bladder top of SD rats were incised and transplanted with the bilayered BMC-gelatin grafts. Similarly, urinary bladders irradiated with 2 Gy once a week for 5 weeks were also conducted. As control, bilayered acellular-gelatin grafts were used. At 4 weeks after transplantation, the bladders were histologically investigated.

Results : At 4 weeks after transplantation into either normal or radiation-injured urinary bladders, incised regions closed. The closed regions of bladder top had reconstructed tissues that were formed with urothelium, and smooth muscle layers. Within the reconstructed tissues, the thickness of the smooth muscle layers in the bilayered BMC-gelatin graft-transplanted bladders were larger compared to controls. The GFP-positive transplanted BMCs were detected. Some of the cells were simultaneously positive for smooth muscle or nerve cell markers.

Conclusion : This study showed that the bilayered BMC-gelatin grafts that were experimentally produced could reconstruct bladder tissues. The grafts would be developed as grafts for bladder augmentation cystoplasty.
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Key words : bladder augmentation cystoplasty, bone marrow-derived mesenchymal cell, gelatin, bladder tissue, rat

I Introduction

In urology, bladder augmentation cystoplasty is conducted to treat a contracted bladder which occurs as a result of various diseases such as neurogenic bladders, radiation cystitis, bladder tuberculosis, or interstitial cystitis[1)-4)]. Bladder augmentation cystoplasty can improve storage function due to enlarged bladder capacity. However, resulting from utilization of a portion of intact ileum[1)], the operation is highly invasive and has several side effects : intestinal stenosis, stone formation, metabolic and electrolyte disturbance, or neoplastic progression[5)]. Thus, in bladder augmentation cystoplasty, developments of novel grafts that can replace the utilization of intact ileum are urgently needed[6)-8)].

Tissue engineering technologies can contribute to produce the novel grafts for bladder augmentation

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cystoplasty[9)-11)]. Previously, we showed that human amnion[12)] or animal tissue-derived membranes[13)] could be used as the grafts for bladder augmentation cystoplasty in rats. However, these membranes have some limitations such as immunological rejection, animal origin, and/or xenobiotics materials in clinical application. To overcome those problems, gelatin sheets that have high biocompatibility and biodegradability have been developed[14)]. In addition, our previous studies indicated that autologous mesenchymal cells derived from bone marrow or adipose tissues can promote reconstruction of bladder tissues[15)-19)]. Thus, a combination of the gelatin sheets and the mesenchymal cells would provide highly efficient grafts for bladder augmentation cystoplasty.

In this study, we experimentally produced 4-type grafts composed of the bone marrow-derived mesenchymal cells (BMCs) and the gelatin sheets, and determined which BMC-gelatin grafts could be developed as clinical grafts to replace the utilization of intact ileum in normal rat urinary bladders. Then, by using our established rat radiation-injured urinary bladders that imitated human radiation cystitis-induced contracted bladder[15)-17)], we determined if the grafts combined with BMCs and gelatin sheets could reconstruct bladder tissues.

Ⅱ Material and Methods

A Animals

Twenty-eight female 10-week-old Sprague-Dawley (SD) rats (Japan SLC Inc., Shizuoka, Japan) were used as recipients. Five male 17-week-old green fluorescence protein (GFP)-transfected SD rats (Tg-SD rats; Japan SLC Inc.) were used as cell donors. All of the rats were maintained with freely available food and water under a 12-hour alternating light-dark cycle. After each experiment, the rats were euthanized by an overdose of pentobarbital sodium solution (Kyoritsu Seiyaku Co., Tokyo, Japan). All animals were treated in accordance with National Institutes of Animal Health Care Guidelines and the guidelines approved by the Animal Ethics Committee of Shinshu University School of Medicine.

B Preparation of bone marrow-derived mesenchymal cells

GFP-transfected Tg-SD rats were anesthetized by intramuscular injection of midazolam (2.0 mg/kg-body weight, Sandoz International GmbH, Tokyo, Japan), medetomidine hydrochloride (0.15 mg/kg-body weight, Kyoritsu Seiyaku Co., Tokyo, Japan), and butorphanol tartrate (2.5 mg/kg-body weight, Meiji Seika Pharma Co., Ltd., Tokyo, Japan), and then further anesthetized by inhalation of 2-3 % sevoflurane (Abbot Japan Co., LTD., Tokyo, Japan). Both femurs of the donor GFP-transfected Tg-SD rats were harvested and both ends were cut, and then bone marrow cells were flushed with 10 ml of culture medium composed of Dulbecco's Modified Eagle Medium (DMEM) high-glucose (Gibco, Thermo Fisher Scientific K.K., Kanagawa, Japan) supplemented with 15 % regular fetal bovine serum (Biowest, Nuaille, France) and 0.1 % penicillin-streptomycin solution (Gibco) from one side of the femurs. After flushing, the cell-solution was centrifuged at 1,000 rpm for 4 minutes, and then the pelleted cells were suspended in the flesh culture medium. After passing through a cell strainer (Life Science, Corning, NY, USA), the suspended bone marrow cells were cultured on type I collagen-coated 10-cm culture dishes (AGC Techo Glass, Shizuoka, Japan) at 37 ℃ in humid air with 5 % CO_2 for 7-10 days. The medium was changed completely every day to wash off unattached cells. We used the attached and proliferating cells on the type I collagen as BMCs[15)-18)].

C Production of bilayered BMC-gelatin grafts

The adherent proliferating BMCs were dissociated with 0.25 % trypsin (Gibco). After suspension at 4.0×10^5 cells/ml in culture medium, the cells were seeded (1 ml) onto temperature-responsive culture dishes (4.0×10^5 cells/ml/well, UpCell® 35-mm dish, CellSeed Inc., Tokyo, Japan), and then Culture medium (2 ml) was added. The cells were cultured for 7-10 days, reaching over-confluence in which more than 80 % of each dish surface was covered by a monolayer of cells.

To harvest the cells after reaching over-confluence, the culture medium was removed, and the temperature of the culture dishes was reduced to 20 ℃. This procedure enables detachment of the BMCs main-

taining cell-cell contacts within the layer itself from the culture dish[16]. The detached BMC layers were then applied to a wetting gelatin hydrogel sheet with dimensions of approximately 12-mm in diameter and approximately 0.5-mm in thickness (gelatin sheet ; Genocel® : dry dimensions of 8-mm in diameter and 0.3-mm in thickness ; Nikke Medical Co., Inc. Osaka, Japan)[14]. Two BMC layer-gelatin sheets, each supporting a BMC layer, were then overlaid together with the cell sides juxtaposed with one another (bilayered BMC-gelatin graft, **Fig. 1A**). In addition, we produced another 3-type grafts ; two acellular-gelatin sheets were overlaid together (bilayered acellular-gelatin graft), the BMC layer-gelatin sheet was not overlaid with another gelatin sheet (monolayer BMC-gelatin graft), and the BMC layer-gelatin sheet was overlaid with an acellular-gelatin sheet (bilayered BMC-acellular gelatin graft). These grafts are indicated in **Fig. 1A**.

The bilayered BMC-gelatin grafts and other type grafts were incubated without culture medium at 37 ℃ in humid air with 5 % CO_2 for 60 minutes. After the incubation, these grafts were cultured with 10 ml R-STEM (Rohto Pharmaceuticals, Osaka, Japan) at 37 ℃ in 5 % CO_2 for 3 days. One of the cultured bilayered BMC-gelatin grafts that were not used in a transplantation (as below) received Masson staining to histologically examine the structural organization **(Fig. 1B)**.

D Production of radiation-injured urinary bladders

Twelve female 10-week-old SD rats were anesthetized as above, and then protected with an iron shield except for a 1-cm diameter circle bordering on the pubic bone to allow radiation of the pelvic region containing the bladder. The exposed area was irradiated with 2 Gy once a week for 5 weeks. Following the last radiation exposure, the rats were maintained for 2 weeks[15)-17)].

E Transplantation of experimental grafts into urinary bladders

Sixteen female 10-week-old SD rats were anesthetized as above, and then their urinary bladder was exposed through a midline lower abdominal incision. The bladder top received a transverse incision, and was divided into anterior and posterior wall, such as a bivalve **(Fig. 1C)**. The experimental grafts : bilayered acellular-gelatin graft, monolayer BMC-gelatin graft, bilayered BMC-acellular gelatin graft or bilayered BMC-gelatin graft were put on the incised region (n =4, in each), and then a biodegradable polyglycolic acid (PGA) nonwoven fabric that was designed to be resorbed after about 2 weeks (Nikke Medical Co., Inc.) was put on the experimental grafts. Then, at the 3, 6, 9, and 12-o'clock position, the bladder and both the experimental graft and PGA nonwoven fabric were sutured with a 5-0 silk thread by mattress suture techniques **(Fig. 1D)**.

Similarly, transplantation of the bilayered BMC-gelatin grafts into the radiation-injured urinary bladders was conducted (n = 5). As control, transplantation of bilayered acellular-gelatin grafts was performed in the radiation-injured urinary bladders (n = 7).

F Histological and immunohistochemical investigations

At 4 weeks after transplantation, the urinary bladders were removed and fixed with 4 % paraformaldehyde solution. Then the samples were embedded in paraffin and cut in 5-μm thick serial longitudinal-sections from bladder top to trigone. For histological investigations, the sections were deparaffinized with xylene, rehydrated with ethanol, and rinsed three times with phosphate-buffered saline (PBS). Each section was stained with Masson trichrome (ScyTek Laboratories, Inc., Logan, UT, USA) according to the protocol of the manufacturer. The Masson trichrome-stained samples were observed and photographed with a common optical microscope. To measure the thickness of smooth muscle layers at both bladder tops that were transplanted with the grafts, and original intact bladder walls that were not incised, the Masson trichrome-stained smooth muscle layers were randomly viewed and measured in 5-10 locations per tissue sample with a x20 objective lens by using image analysis software (cellSens, Olympus Co., Tokyo, Japan).

For immunohistochemistry, some of the deparaffinized sections were immersed in 10 mM sodium citrate (pH 6.0) and microwaved at 100 ℃ for 10 minutes for antigen retrieval. The sections were blocked

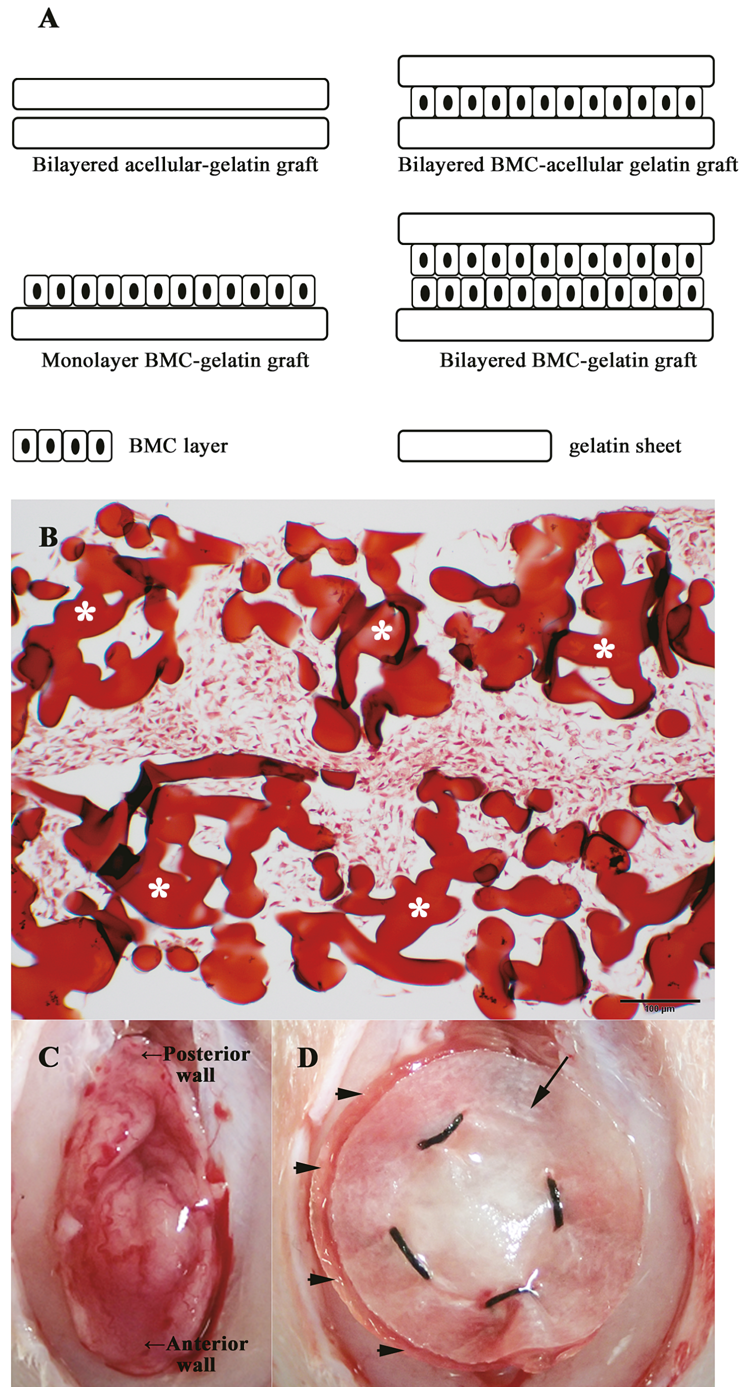

Fig. 1 Production of 4-type experimental grafts and transplantation into urinary bladders. (A) This study experimentally produced 4-type grafts, bilayered acellular-gelatin graft ; two acellular-gelatin sheets were overlaid, monolayer BMC-gelatin graft ; a BMC layer-gelatin sheet was not overlaid with a gelatin sheet, bilayered BMC-gelatin graft ; a BMC layer-gelatin was overlaid with an acellular gelatin sheet, bilayered BMC-gelatin sheet ; two BMC layer-gelatin sheets were overlaid together with the cell sides juxtaposed with one another. (B) At 3 days after overlaying, the BMC layers between gelatin sheets tightly attached to gelatin fibers (asterisks), and fully contacted each other. Bar : 100μm. (C) Just prior to transplantation, urinary bladders received a transverse incision, and were divided into anterior and posterior wall. (D) For transplantation, the experimental sheets (arrowheads) were put on the incised region, and then a PGA nonwoven fabric (arrow) was put on the sheets.

with 1.5 % non-fat milk in PBS for 1 hour at 4 ℃. Each section was incubated with GFP-antibody (1 : 1000, goat polyclonal, abcam, Cambridge, UK) to detect the BMCs, and smooth muscle actin (SMA, 1 : 100 mouse monoclonal ; Progen Biotechnik GmbH, Heidelberg, Germany) to identify mature smooth muscle cells, desmin (1 : 150 rabbit polyclonal ; Progen Biotechnik GmbH) a marker for immature smooth muscle cells, or Pax7 (1 : 1000, rabbit polyclonal ; Lifespan Biosciences, Inc., Seattle, WA, USA) as a marker for myoblasts, or calcitonin gene-related peptide (CGRP, 1 : 500, guinea pig polyclonal, Progen Biotechnik GmbH) as a marker for afferent nerve cells for 12 hours at 4 ℃. After the primary antibody reactions, the sections were incubated with the respective secondary antibodies consisting of donkey anti-goat conjugated with Alexa Fluor 488 (1 : 250 ; Molecular Probes, Eugene, OR, USA) and donkey anti-mouse, rabbit, or guinea pig each conjugated with Alexa Fluor 594 (1 : 250 ; Molecular Probes) for 1 hour at 4 ℃. After rinsing, they were counterstained with 5 μg/ml 4', 6-diamidino-phenylindole dihydrochloride (DAPI ; Molecular Probes). The immunohistochemically stained samples were observed with a fluorescence microscope (Keyence, Osaka, Japan).

G Statistical analysis

Results were expressed as means ± standard deviations. Statistical differences were determined using the Excel® Statistics program (Esumi Co., Ltd. Tokyo, Japan). Comparisons were made by non-repeated measures analysis of variance (ANOVA) or t-tests. P-values less than 0.05 were considered statistically significant.

Ⅲ Results

A Histological investigations of bilayered BMC-gelatin grafts

Masson stain samples of bilayered BMC-gelatin graft were observed. Between the gelatin sheets, the BMC layers tightly attached to gelatin fibers, and fully contacted each other. Some of the BMCs migrated along the gelatin fibers (see **Fig. 1B**).

B Bladder tissues reconstructed by transplantation of 4-type experimental grafts into normal urinary bladders

At 4 weeks after transplantation, incised regions that were treated with bilayered acellular-gelatin **(Fig. 2A)**, monolayer BMC-gelatin **(Fig. 2B)**, bilayered BMC-acellular gelatin **(Fig. 2C)**, or bilayered BMC layer-gelatin **(Fig. 2D)** grafts were well closed, and the treated bladders were similar in appearance to the pre-operated bladders. While small fragments of PGA nonwoven fabric or thread were still present within the bladder lumen, at the closed regions of bladder tops, those experimental grafts were replaced by reconstructed tissues. The reconstructed tissues were formed with urothelium arranged into a multi-layered form, smooth muscle layers integrated into small cluster form, and filamentous gelatin fiber-like materials that might be derived from biodegraded gelatin sheets **(Fig. 2E-H)**. The urothelia were similar to the normal ones, and did not show any differences among the experimental grafts **(Fig. 2E-H)**. The thickness of the smooth muscle layers within the tissues of bladder top transplanted with the bilayered BMC-gelatin grafts was the greatest compared to the tissues transplanted with the bilayered acellular-gelatin, the monolayer BMC-gelatin, or the bilayered BMC-acellular gelatin graft **(Table 1)**. Thus, this study made a decision to investigate the effects of the bilayered BMC-gelatin grafts in radiation-injured urinary bladders.

C Bladder tissues reconstructed with bilayered BMC-gelatin grafts into radiation-injured urinary bladders

At 4 weeks after transplantation into radiation-injured urinary bladders, incised regions that were transplanted with control bilayered acellular- **(Fig. 3A)** or bilayered BMC- **(Fig. 3B)** gelatin grafts were closed. While small fragments of PGA nonwoven fabric or thread were still present within the bladder lumen, at the closed regions of bladder tops, either the control grafts **(Fig. 3C)** or the bilayered BMC-gelatin grafts **(Fig. 3D)** were replaced by reconstructed tissues that were formed with urothelium, smooth muscle layers, and filamentous gelatin fiber-like materials. These

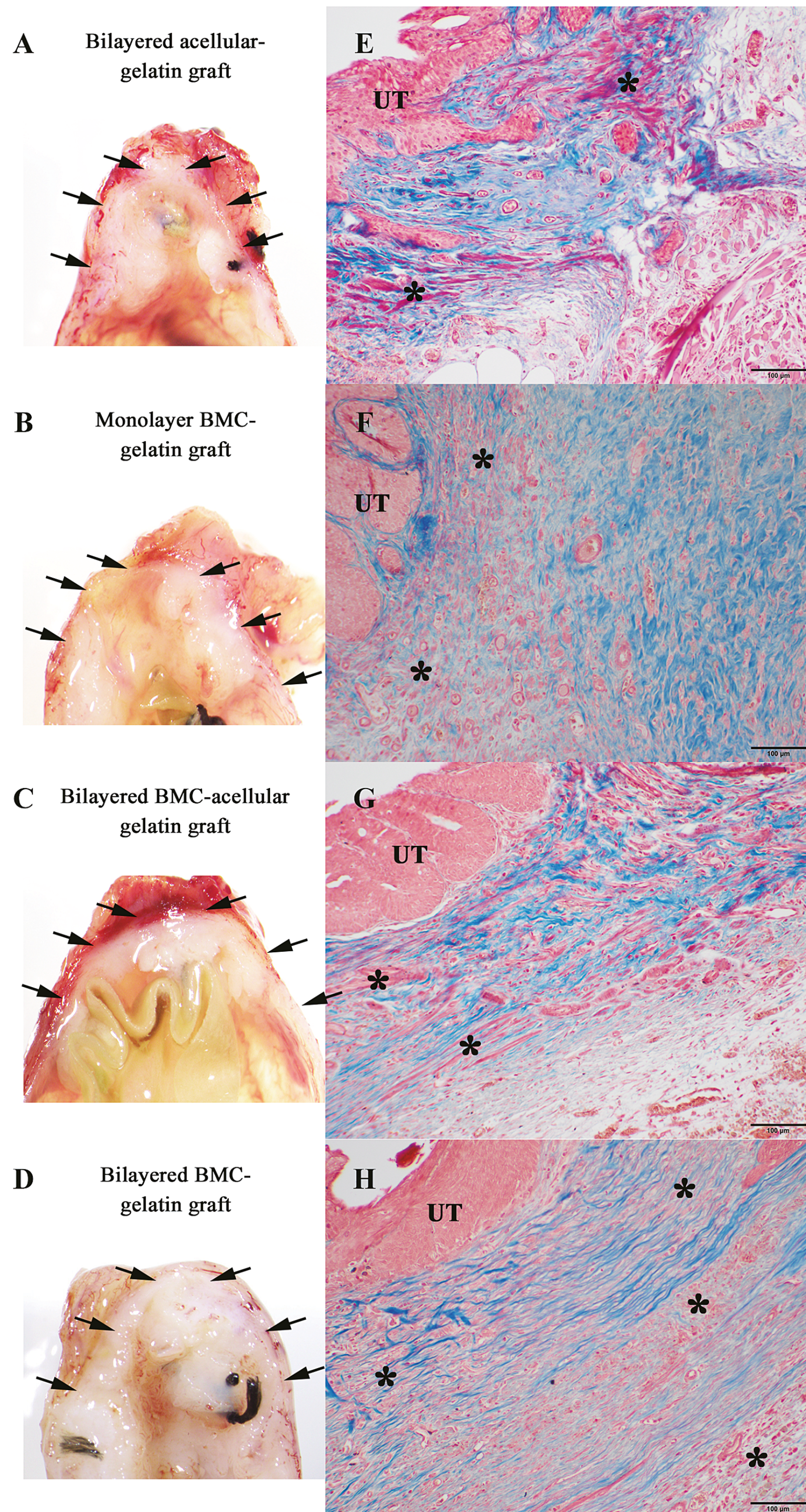

Fig. 2 At 4 weeks after transplantation of experimental sheets into normal urinary bladders. (A-D) Incised regions transplanted with bilayered acellular-gelatin (A), monolayer BMC-gelatin (B), bilayered BMC-acellular (C), or bilayered BMC-gelatin (D) grafts were well closed. These transplanted experimental grafts were replaced by reconstructed tissues (arrows). (E-H) At the closed regions of bladder top, the tissues reconstructed with bilayered acellular-gelatin (E), monolayer BMC-gelatin (F), bilayered BMC-acellular (G), or bilayered BMC-gelatin (H) grafts were formed with urothelium (UT), smooth muscle layers (asterisks), and filamentous gelatin fiber-like materials (blue). Bars : 100μm.

Table 1 Thickness of smooth muscle layers within reconstructed tissues of bladder tops at 4 weeks after transplantation of experimental grafts into normal urinary bladders

	Bilayered acellular-gelatin graft	Monolayer BMC-gelatin graft	Bilayered BMC-acellular gelatin graft	Bilayered BMC-gelatin graft
Smooth muscle layers (μm)	50.59 ± 5.82	53.78 ± 9.14	49.63 ± 1.21	164.54 ± 13.93* § †

*P＜0.01 ; compared to bilayered acellular-gelatin graft, § P＜0.01 ; compared to monolayer BMC-gelatin graft,
† P＜0.01 ; compared to bilayered BMC-acellular gelatin graft.

results were similar to the transplantation into normal urinary bladders. At the closed regions of bladder tops, the thickness of the smooth muscle layers within the tissues reconstructed with the bilayered BMC-gelatin grafts was greater than that reconstructed with the control bilayered acellular-gelatin grafts **(**P＜0.01, **Fig. 3E)**. In addition, the rate of smooth muscle layer thickness to origin recipient ones within the intact bladder walls in the bilayered BMC-gelatin graft-transplanted bladders was higher than that in the control graft-transplanted bladders **(**P＜0.01, **Fig. 3F)**.

D Survival and differentiation of transplanted BMCs

At the 4 weeks closed regions of bladder tops, GFP-positive transplanted BMCs, which formed the monolayer BMC-gelatin, bilayered BMC-acellular-gelatin, or bilayered BMC-gelatin grafts, were detected in the reconstructed tissues (data not shown). These results showed that the BMCs survived in the recipient bladders.

In the radiation-injured urinary bladders, the GFP-positive transplanted BMCs that formed the bilayered BMC-gelatin grafts were present in the reconstructed tissues at the closed regions of bladder tops **(Fig. 4A-D)**. In the same observed position in each, there were also SMA-positive mature smooth muscle cells **(Fig. 4E)**, desmin-positive immature smooth muscle cells **(Fig. 4F)**, Pax7-positive myoblasts **(Fig. 4G)**, or CGRP-positive afferent nerve cells **(Fig. 4H)**. Some of the transplanted GFP-positive BMCs were simultaneously positive for these makers **(Fig. 4I-L)**.

In contrast, the reconstructed tissues had numerous GFP-negative and smooth muscle markers-positive cells that were derived from recipient tissues. In addition, the incised regions transplanted with control bilayered acellular-gelatin grafts were also closed, and then the control closed regions similarly had reconstructed tissues. These results showed that the reconstructed tissues were formed with not only the transplanted BMCs, but also the cells derived from the origin recipient bladder tissues.

Ⅳ Discussion

Previously, we showed that the bone marrow-derived cells had the potential to reconstruct functional urinary bladders. To deliver the cells into recipient bladders, we performed two methods, the direct-injection of single cells[17)-19)] and the patch-transplantation of cell sheets[16)]. However, these methods cannot be applied for bladder augmentation cystoplasty. Thus, this study attempted to develop based-techniques of grafts combining both the mesenchymal cells and the gelatin sheets for bladder augmentation cystoplasty.

This study experimentally produced 4-type grafts composed of BMCs and the gelatin sheet **(**see **Fig. 1A)**. We overlaid two BMC layer-gelatin sheets together with the cell sides juxtaposed with one another. The 3D-conformation provides naturally occurring cell-to-cell contacts, and each cell can increase its activation[15)]. In contrast, over 3-layers cannot supply enough nutritious substances and oxygen to the cells arranged within inside-center regions. To estimate those experimental grafts, we transplanted each type of graft onto incised bladder top in normal or radiation-injured urinary bladders. Four weeks later, the bilayered BMC-gelatin graft-transplanted bladders showed the greatest thickness of smooth muscle layers among the other experimental grafts-transplanted bladders. Thus, the reconstructed tissues in the bilayered

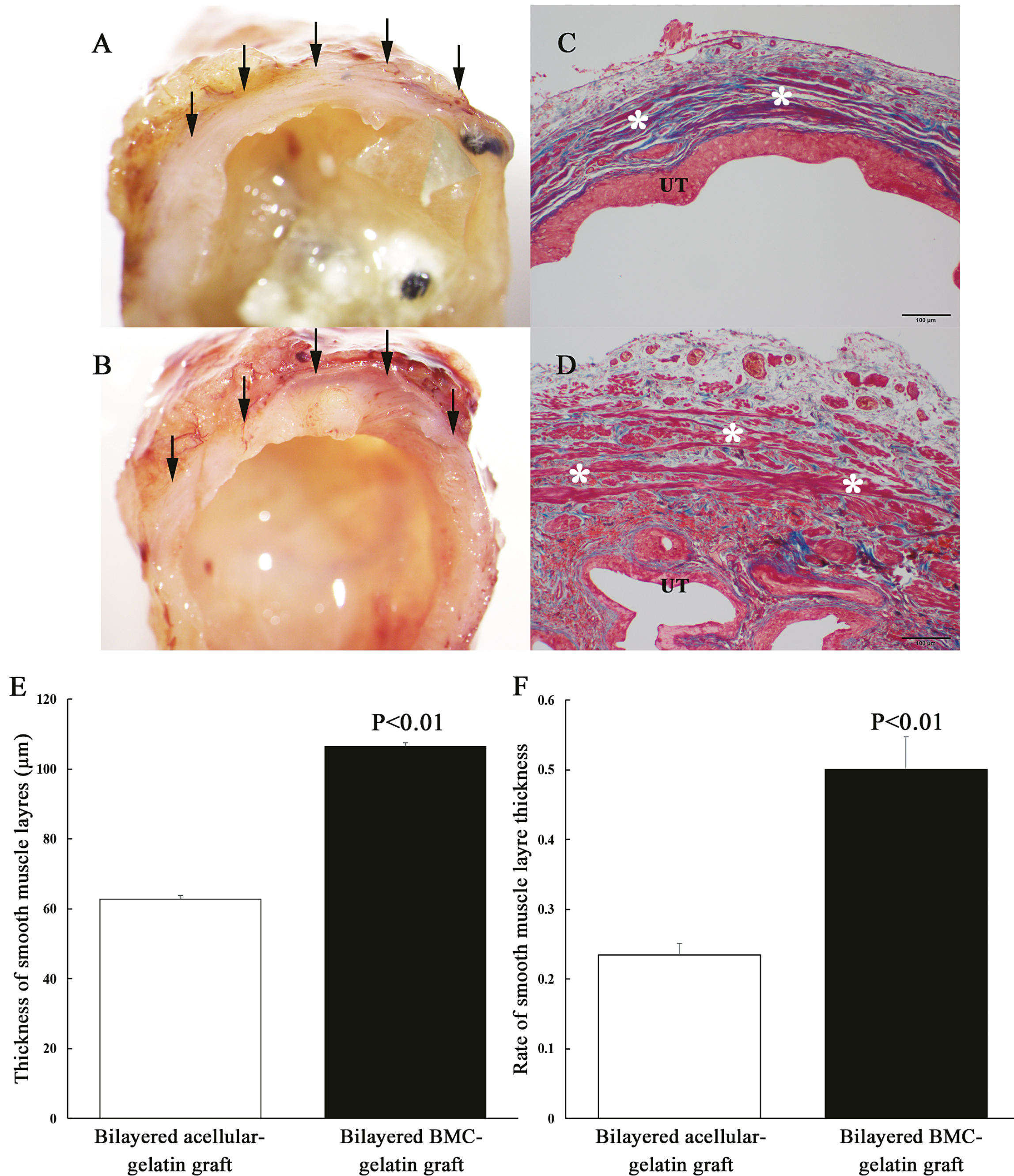

Fig. 3 At 4 weeks after transplantation of bilayered BMC-gelatin grafts into radiation-injured urinary bladders. (A and B) Incised regions transplanted with control bilayered acellular- (A) or bilayered BMC- (B) gelatin grafts were closed. The closed regions of bladder top had reconstructed tissues (arrows). (C and D) The reconstructed bladder tissues with control bilayered acellular- (C) or bilayered BMC- (D) gelatin grafts were formed with urothelium (UT), smooth muscle layers (asterisks), and filamentous gelatin fiber-like materials (blue). Bars : 100μm. (E) At the bladder top, thickness of the smooth muscle layers of the bilayered BMC-gelatin graft-transplanted bladders was greater than that of the control bilayered acellular-gelatin graft-transplanted bladders ($P<0.01$). (F) Rate of smooth muscle layer thickness to origin recipient ones within the intact walls in the bilayered BMC-gelatin graft-transplanted bladders was higher than that in the control bilayered acellular-gelatin graft-transplanted bladders ($P<0.01$).

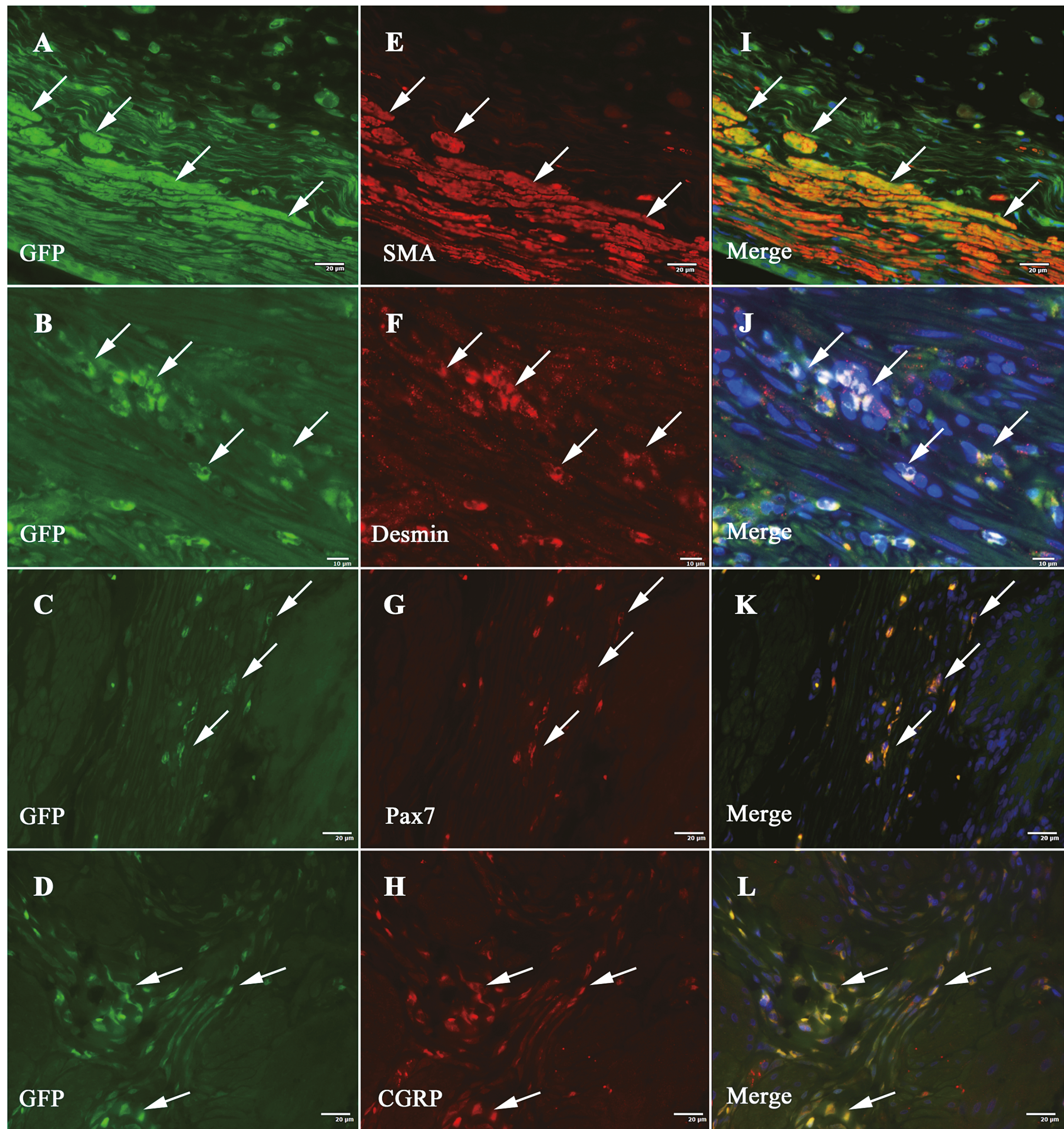

Fig. 4 Differentiation of transplanted BMCs. (A-D) GFP-positive transplanted BMCs (green) were present within the reconstructed tissues. (E-H) In the same observed position in each, SMA- (E, red), desmin- (F, red), Pax7- (G, red), or CGRP- (H, red) positive cells were present. (I-L) Some of the GFP-positive BMCs were simultaneously positive for these marker antibodies (arrows, yellow). Bars : 10 or 20μm.

BMC-gelatin graft-transplanted bladders tended to be thicker than those of the other control graft-transplanted bladders.

Here, this study histologically investigated the mechanisms of these tissue recoveries. Within the reconstructed tissues at the bladder top, GFP-positive transplanted BMCs were present. Some of the GFP-positive BMCs differentiated into SMA-positive mature smooth muscle cells, desmin-positive immature smooth muscle cells, Pax7-positive myoblasts, or GFP-positive afferent nerve cells. These results showed that the bilayered BMC-gelatin grafts might contrib-

ute as cell sources. Our previous study showed that BMC monolayer-sheets could provide 24 kinds of growth factors and/or cytokines that were muscle tissue development-, peripheral nerve system development-, angiogenesis-, apoptosis regulator-, cell differentiation-, morphogenesis-, immune response-, and ureteric bud development-related bioactive substances in radiation-injured bladders[16]. Thus, these growth factors and/or cytokines provided from BMC layers might also promote tissue recovery. In addition, GFP-negative and SMA-positive cells were also present within the reconstructed tissues of the bladder top. Thus, it is likely that these cells migrated from origin recipient tissues. In contrast, the control rats receiving the control bilayered acellular-gelatin grafts also showed the reconstruction of tissues. This suggested that the recipient cells might have migrated through the gelatin sheets. These results showed that the reconstructed tissues were formed with not only the transplanted BMCs, but also the cells migrated from origin recipient tissues. Therefore, the grafts combined with both BMC layers and gelatin sheets could be highly effective in reconstructing bladder tissues.

To develop the bilayered BMC-gelatin grafts for clinical use, we need to overcome some limitations. In this study, we provided strength to suture the experimental grafts on incised region of bladders with PGA nonwoven fabric. While the PGA nonwoven fabrics were designed to be resorbed after about 2 weeks in the body, small fragments of PGA nonwoven fabric were still present within the bladder lumen of almost all recipient rats. Thus, we would need to develop a replacement for the PGA nonwoven fabric, or increase the mechanical strength of the gelatin sheets. The main aims of bladder augmentation cystoplasty are enlargement of both bladder capacity and compliance ; however, this study did not measure these functions in the graft-transplanted rats. To measure these parameters, cystometric investigations in which a catheter is inserted from the bladder top are needed. Because this study histologically focused on the bladder tops, the cystometric investigations were not conducted. As then next steps, we will use larger animals such as rabbits, and conduct measurements of leak point pressure that are able to estimate the bladder capacity and compliance without inserting the bladder catheter.

In the process to clinical use, the bilayered BMC-gelatin grafts have some advantages. One is easy scale-up to human size by using large gelatin sheets. Secondly, this bilayered BMC-gelatin graft is able to be produced with simple handling. Thirdly, compared to collagen, the gelatin is generally considered to have a high migration of cells. Finally, while the universal collagen-based materials were extracted with a low-temperature acid solution, the gelatin hydrogel sheets that were used in this study were produced through alkali-thermal decomposition. Thus, manufacturing processes of the gelatin sheets can avoid contamination risks of infection or toxicity compared to these of collagen sheets[14,20]. These advantages might contribute to developing the bilayered BMC-gelatin grafts in future.

V Conclusions

Bilayered BMC-gelatin grafts were produced by overlaying two BMC layer-gelatin sheets. At 4 weeks after transplantation into normal or radiation-injured urinary bladders, incised regions transplanted with the bilayered BMC-gelatin grafts were closed with reconstructed tissues that were formed with urothelium, smooth muscle layers, and filamentous gelatin fiber-like materials. Also, the thickness of the smooth muscle layers in the bilayered BMC-gelatin graft-transplanted bladders was the greatest compared to other control graft-transplanted bladders. Some GFP-positive transplanted BMCs differentiated into smooth muscle- or afferent nerve-marker positive cells. These results showed that the bilayered BMC-gelatin grafts could reconstruct the bladder tissues.

Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest to disclose.

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