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# Bone Marrow–Derived Cells Regenerate Structural and Functional Lower Urinary Tracts

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Additional information is available at the end of the chapter

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## 1. Introduction

In this chapter, we show that with the application of tissue engineering principles, utilization of bone marrow-derived cells has the potential to reconstruct functional lower urinary tracts, which are composed mainly of the urinary bladder [1] and urethra [2]. Patients complain about lower urinary tract dysfunctions that significantly decrease their quality of life. Regenerative medicine provides great hope for the recovery of lost tissue and organ functions. In urology, novel regenerative medicine techniques are being developed for the treatment of irreversibly damaged lower urinary tracts. Notably, injection of autologous cells harvested from adipose tissue into the sphincter and urethra has been attempted clinically to treat urinary incontinence by increasing the urethral closure pressure [3]. Our laboratory has also been vigorously investigating regenerative medicine as a tool to treat irreversibly damaged urinary bladders and urethras.

An important factor in the development of regenerative medicine is selection of the proper source for the regenerative cells and/or tissues. Recently, attempts to use various kinds of cells, such as induced pluripotent stem cells, embryonic stem cells, and mesenchymal cells derived from adipose and oral mucosal tissues, have been reported. Based on the literature, we have considered many sources of cells from which to derive adult somatic stem cells that could regenerate lower urinary tracts [4-7]. Recently, we have focused on two sources of cells with the potential to meet a variety of demands: bone marrow-derived cells [8] and adipose-derived cells [9]. In this chapter, we show that cells derived from bone marrow are an excellent resource for the development of regenerative medicine. These cells are capable of differentiating both in vitro and in vivo along multiple pathways that include striated and smooth muscle [10-16] as well as bone, cartilage, adipose, neural cells, tendon, and connective tissue [17,18]. Also, bone marrow-derived cells, which are easy to grow in culture, produce cytokines and growth

factors that accelerate healing in damaged tissues and inhibit apoptosis and the development of fibrosis [19,20]. However, the operation to harvest the bone marrow cells is generally considered to have higher patient risks compared to harvesting adipose cells. The increased risk for harvesting bone marrow cells for autologous transplantation is especially important for elderly patients with lower urinary tract symptoms (LUTS) and who may have other diseases as well.

Equally important as the sources of cells for regenerative medicine are the survival rates for implanted cells, the differentiation into target cell types, and the structural support that enables the reconstruction within the recipient tissues [21]. The survival, differentiation, and reorganization of the implanted cells are affected by the microenvironment within the recipient tissues. However, our understanding of these microenvironments is currently insufficient to provide clinically effective and reliable resources for regenerative medicine. Thus, to obtain the optimum microenvironment, we need to investigate the utilization of scaffolds, growth factors, and combinations of these materials.

This chapter has three major topics: (1) implantation of allogenic mouse bone marrow-derived cells into freeze-injured urinary bladders, (2) implantation of autologous bone marrow-derived cells into freeze-injured urethral sphincters, and (3) importance of the microenvironment in reconstructing functional lower urinary tracts. We show that bone marrow-derived cells implanted into freeze-injured bladders or urethras differentiate into smooth muscle and striated muscle cells. These cells become organized into layered structures that are associated with the recovery of tissue function. In injured tissues, we have begun to uncover the important roles that may support the differentiation. Our information leading to future studies will enable the development of regenerative medicine in urology and other clinical areas.

## **2. Reconstruction of functional urinary bladders**

Our final goal is to develop treatments for irreversibly damaged urinary bladders resulting from neurogenic bladder associated with brain and spinal cord disease, cystitis, peripheral neuropathy, or radiotherapy-induced injury. To determine the capacity of bone marrow-derived cells to fulfill these goals, we have performed preliminary investigations in allogeneic transplantation by using a mouse freeze-injured urinary bladder model [1].

### **2.1. Freeze-injured urinary bladder model**

Three days prior to implantation, we induce a highly reproducible freeze-injury to the urinary bladders of mice [1]. We apply an iron bar (25 × 3 × 2 mm) chilled by dry ice onto the posterior urinary bladder wall for 30 seconds. Placement of the chilled iron bar causes local freezing of the bladder wall tissues. Within 10 seconds after removal of the bar, the frozen spot thaws due to body and/or room heat and appears to the naked eye similar to the intact normal bladder wall. However, when we monitor blood flow within the capillaries of the frozen area with a charged-couple device (CCD) video microscopy, the local circulation pauses for approximately 20 min after the operation, and then it resumes. It is likely that the freeze-injured urinary

bladders experience a period of ischemia followed by reperfusion as described by one of the microcirculation dysfunction models [22]. At 3 days after the freeze-injury operation, the wounded area, which occupies approximately one-third of each urinary bladder, is readily identified by the presence of a hematoma. The freeze-injured urinary bladders have both injured and uninjured regions that are easily observed by histology. The smooth muscle layers of the injured regions are disorganized and readily distinguished from the surrounding uninjured regions (Figure 1A).

## **2.2. Implantation of cultured bone marrow-derived cells**

We harvest mouse bone marrow cells by flushing them out from both ends of femurs, and the recovered cells are cultured on collagen-coated dishes for 7 days. During the culture period, we completely replace the 15% fetal bovine serum-containing medium every day and remove non-attached cells. Immediately after plating in dishes, the bone marrow cells consist of heterogeneous, spindle-shaped, round, and polygonal cell types along with red blood cells. At 5 days after seeding, the cells achieve approximately 80% confluence, and at that time we transfect them with the green fluorescence protein (GFP) gene for identification in the recipient tissues. After 7 days of culture (i.e., 2 days after transfection), the adhered proliferating cells are relatively homogenous in spindle shaped appearance, and they expressed GFP. We confirm that the cultured cells do not differentiate into smooth muscle cells during the culture period.

The culture conditions readily promote attachment and proliferation of bone marrow cells. The cultured bone marrow-derived cells contain a variety of stem cell types, such as hematopoietic, mesenchymal, and stromal stem cells [17,18]. Marker proteins on cells can be used to sort the ones that will differentiate into specific target cells [23-27]. However, which of the sorted cells are best for clinical use is unknown [28]. The simplicity of our selection procedure, based only on attachment and proliferation of bone marrow cells on collagen, would be a significant advantage for clinical applications.

On Day 7 of culture, we dissociate the cultured bone marrow-derived cells and allotransplant  $2.0 \times 10^6$  cells with a 30-gauge micro-syringe into the center of the injured region of the 3-day-old freeze-injured urinary bladders. As controls, we inject cell-free solution. The implantation cell number and volume are chosen to avoid further damaging the cells with shear stress or the recipient tissues by bursting the bladder wall. Each operation is performed under a stereomicroscope where we visually confirm the presence of a small swelling, indicating that the implanted cells remain at the site.

## **2.3. Reconstructed smooth muscle layers**

At 14 days after implantation, we estimate the effect of the bone marrow-derived cells. The regions implanted with these cells have numerous alpha-smooth muscle actin (SMA)-positive smooth muscle cells compared to the control regions injected with the cell-free solution (Figure 1B). The cells are organized into distinct smooth muscle layers. In contrast, the few SMA-positive cells present in the regions injected with the cell-free solution are not organized into layers.

At 14 days, SMA mRNA expression in the implanted regions is significantly higher than that in the cell-free injected regions, which supports the immunohistochemical observations [1]. In fact, the expression level of SMA mRNA in the implanted regions is not significantly different from that in the normal urinary bladder. Expression levels of other smooth muscle cell differentiation marker genes in the implanted region are also elevated. In the implanted regions, smooth muscle myosin heavy chain (MHC) and calponin I mRNA expression are significantly higher than in the cell-free injected control regions [1]. There are no significant differences in MHC and calponin I mRNA expression levels when the implanted regions are compared to the normal urinary bladders. Desmin mRNA expression is significantly higher than either control or normal regions. Thus the implanted regions have a larger number of mature smooth muscle cells and developing smooth muscle layers compared to the control regions [29-33]. Collectively, the immunohistochemical and gene expression results show that the implanted regions have formed smooth muscle layers composed of regenerated smooth muscle cells during the 14 days of the study period, while the control regions have only minimal recovery.

#### **2.4. Progress of implanted bone marrow-derived cells**

We detect the implanted bone marrow-derived cells in the recipient tissues by the presence of GFP labeling. Some of the GFP-labeled implanted cells are positive for proliferating cell nuclear antigen (PCNA), a marker of proliferating cells. In addition, within

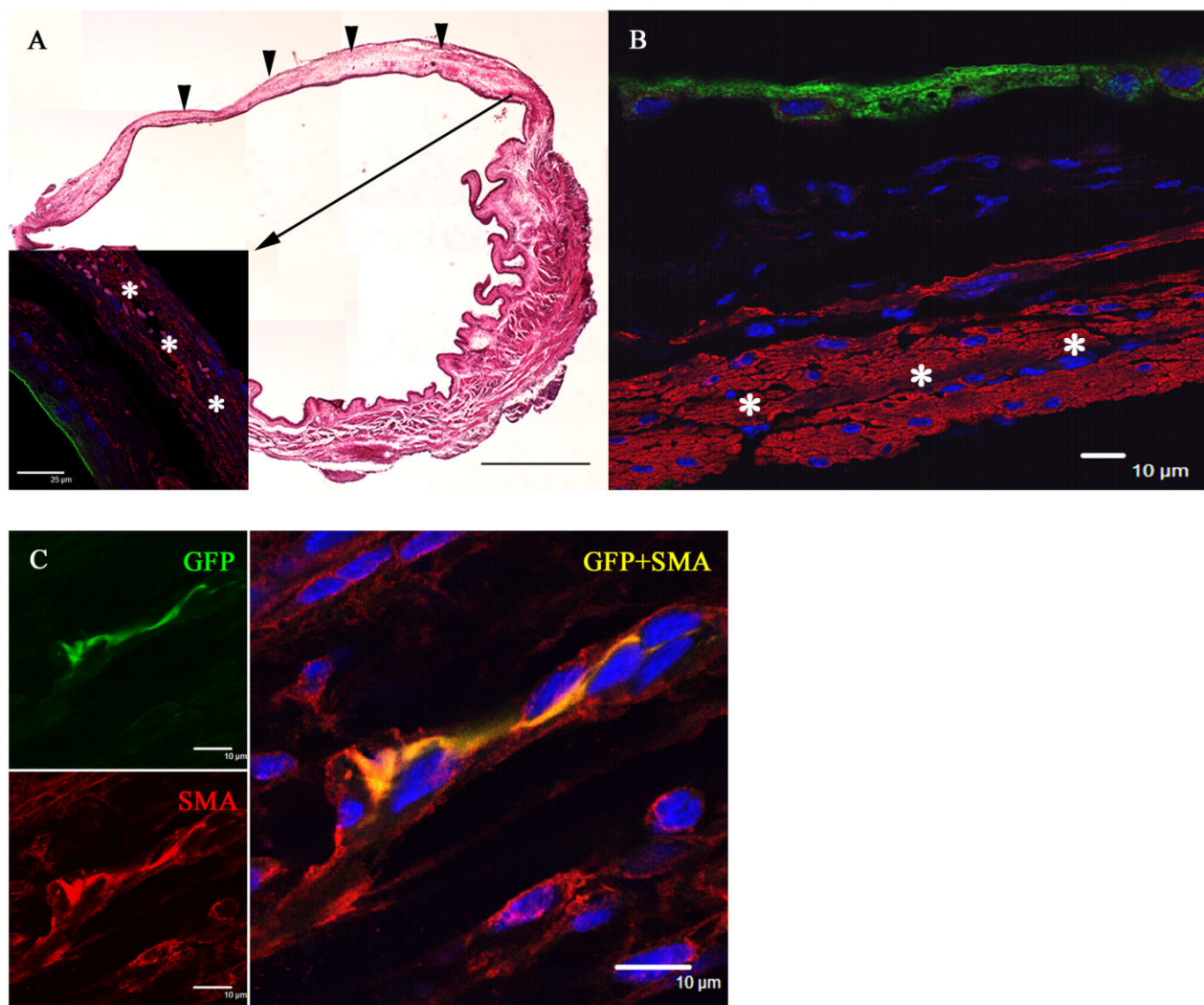
the newly formed smooth muscle layers, some GFP-labeled cells that do not express smooth muscle cell differentiation markers are organized into cord-like structures. The cells may have differentiated into cell types that provide histoarchitectural elements for the blood vascular system [24] or the nervous system [25]. The implanted bone marrow-derived cells may participate in as yet other unknown changes in the various tissues of the urinary bladder.

We perform double staining with the smooth muscle cell differentiation markers and GFP antibody to identify the regenerated smooth muscle cells that are derived from the implanted cells. Both GFP- and SMA-positive cells show that the implanted bone marrow-derived cells differentiate into smooth muscle cells within the injured urinary bladders (Figure 1C). Other implanted GFP-labeled cells are also positive for MHC, desmin, and calponin I. Some of the GFP-labeled, differentiated smooth muscle cells contact each other, and they also contact non-GFP-labeled smooth muscle cells of the host that surround the implanted regions. Collectively, these cells form smooth muscle layers. The differentiation toward smooth muscle cells occurs after implantation because none of the cells expressed detectable levels of the marker proteins while in culture.

#### **2.5. Recovery of bladder contractions**

Cystometric investigations at 3 days after injury show that the mice do not have defined regular bladder contractions. The bladder contractions at 14 days after cell-free control injection also remain disrupted. However 14 days after cell implantation, there are distinct regular bladder contractions that are similar to those of normal mice without injury [1]. Thus, cystometric





**Figure 1.** Implantation of bone marrow-derived cells into freeze-injured urinary bladders. (A) At 3 days after operation, the freeze-injured regions (arrowheads and asterisk in inset) lose typical smooth muscle layers. Black bar: 1 cm. (B) At 14 days, the bone marrow-derived cell-implanted regions have numerous SMA-positive smooth muscle cells (red, asterisks) that are organized into layers (green, urothelium; blue, nuclei). (C) The cells detected with GFP antibody (upper left inset, green cells) are simultaneously positive for the smooth muscle marker SMA (bottom left inset, red cells) within the newly formed smooth muscle layers. The merged images (right, yellow cells) show that the implanted GFP-labeled cells have differentiated into cells expressing smooth muscle markers. Blue, nuclei.

investigations indicate that implanted bone marrow-derived cells have the potential to restore some or all normal bladder functions. We believe that the smooth muscle layers reconstructed by the implantation of the cells contribute to the restoration of bladder contractions.

### 3. Reconstruction of functional urethral sphincters

In clinical diagnosis, urinary incontinence is separated into two major categories: (1) stress urinary incontinence and (2) post-surgical urinary incontinence associated with intrinsic sphincter deficiency (ISD). Stress urinary incontinence is related to urethral hypermobility,

which results from the loss of bladder neck support. This form of urinary incontinence can be improved by surgical therapies to lift the bladder and urethra. In contrast, post-surgical ISD-related urinary incontinence can occur as a result of radical prostatectomy or bladder neck surgery. It is characterized by severely decreased urethral closure pressure due to malfunction of the closure mechanism, and it results in intractable urinary incontinence. Improvement of urethral closure pressure is widely accepted as one of the effective treatments for ISD-related urinary incontinence [34]. In the urinary continence system, the urethral sphincter is composed of both striated and smooth muscle cells and produces urethral closure pressure. Thus, our strategy for relieving ISD-related urinary incontinence is the reconstruction of functional urethral sphincters by the implantation of autologous bone marrow-derived cells [2].

### 3.1. Freeze-injured urethral sphincter model

For ISD-related urinary incontinence studies, we have developed a rabbit freeze-injured urethral sphincter model [2]. The sphincter, which is located at the internal urethral orifice where it joins the inferior end of the bladder, is sprayed with the liquid nitrogen for 15 sec. The frozen regions are thawed by room and body heat within approximately 20 sec. As an immediate consequence of the freeze and thawing, the wounded internal urethral orifice is flaccid and gapes open.

Seven days later we compare the effect of the urethral freeze/thaw procedure to sham-operated uninjured animals. In sham-operated animals with uninjured urethral sphincters, the internal urethral opening remains tightly closed [2]. The muscle tissues within the intact sphincters are composed of striated muscle containing highly organized myofibrils and smooth muscle cells containing irregularly placed myofibrils. Immunohistochemical analysis shows that the sham-operated urethral sphincters are composed of distinct muscle tissues containing numerous myoglobin- and SMA-positive cells. In contrast, the freeze-injured internal urethral openings remains flaccid (Figure 2A), and the leak point pressure of the injured animals is significantly lower than that of the sham-operated animals. Consistent with these observations, the injured urethral sphincters show reactive changes including loss of muscle mass and relative disorganization of the remaining muscle tissues (Figure 2A). The majority of the striated and smooth muscle cells are lost, and there is a complete absence of most myoglobin- and SMA-positive cells (Figure 1A).

Our ISD-related urinary incontinence model is similar to other models of urinary incontinence with lost striated and smooth muscle and reduced leak point pressures [35–38]. The urinary sphincters of patients with post-surgical urinary incontinence are irreversibly damaged. However, this appears not to be the case in our model. The cell-free treated rabbits show a weak but natural recovery of striated and smooth muscle cells associated with a slight increase of leak point pressure. Rabbits may have inherently different regenerative powers than humans. Additionally, the rabbits are young and in good health, in contrast to patients with ISD-related urinary incontinence, who are typically elderly and not in good general health. In our rabbit model, we intentionally avoided more severe and serious sphincter damage that would have produced irreversible incontinence because of the potential for urethral stricture

or perforation, followed by death. Thus, our model is considered to be an acute incontinence of relatively short duration [2].

### **3.2. Implantation of autologous bone marrow-derived cells**

To conduct autologous implantation without euthanasia, we harvest bone marrow cells from a femur of each anesthetized animal by the flush out method, which is modified from the technique described by Kushida et al. [39]. Two pediatric bone marrow needles are inserted 2 cm apart into a femur, and then the cells are flushed out with saline pushed through one needle and collected in a tube through the other needle. The harvested bone marrow cells are cultured on type I collagen-coated culture flasks for 10 days. The culture and cell-labeling methods are the same as for mouse bone marrow-derived cells (as above 1.2). During the culture, the cytomorphic changes are similar to those in the mouse bone marrow-derived cells [2]. At 10 days, the cultured cells express mesenchymal cell marker STRO1 (CD34), but not myoglobin, SMA, or Pax7, which are differentiation markers for striated muscle cells, smooth muscle cells, and myoblast, respectively.

Aging, disease processes, and medications may affect the potential of bone marrow cells for differentiation. Thus, for the purpose of advancing the fundamental research necessary for understanding the basic parameters of autologous bone marrow-derived cell growth, differentiation, and transplantation, we selected young and healthy rabbits. The large size of these animals, in contrast to rats, mice, or other rodents, facilitates the performance of the autologous bone marrow-derived cell-implantation procedures.

Ten days after culture, and 7 days after the freeze-injury operation, we implant  $0.5 \times 10^6$  autologous bone marrow-derived cells suspended in 100  $\mu$ l culture medium. A total of  $2.0 \times 10^6$  cells are injected via a 29-gauge syringe needle into the injured regions at the 3-, 6-, 9-, and 12-O'clock positions. For controls, we inject cell-free solution with same manner. The implantation cell number and volume are chosen for the same reasons described above (section 1.2).

### **3.3. Reconstructed layered muscle structures**

At 7 and 14 days after cell-implantation or cell-free control injection, recovery of the urethral sphincters is determined by histology, cytology, and immunohistochemistry [2]. At 7 days after the cell-free control injection, there are few myoglobin-positive striated muscle cells, and few clusters composed of SMA-positive smooth muscle cells. In contrast, at 7 days after cell implantation, there are developing muscle layers composed of myoglobin-positive striated cells, and clusters composed of SMA-positive smooth muscle cells. At that time, the proportions of the myoglobin- and SMA-positive areas in the cell-implanted regions are significantly higher than in the cell-free injected regions [2].

At 14 days after control cell-free injection, the regional composition of cells is similar to the 7-day control regions with relatively few cells expressing myoglobin or SMA [2]. In contrast, at 14 days after cell implantation, the regions have distinctly regenerated muscle layers composed of numerous myoglobin-positive striated and SMA-positive smooth muscle cells that are



similar to the intact urethral sphincters (Figures 2B, C). At that time, the proportion of both myoglobin- and SMA-positive areas are significantly higher than in the control regions.

Bone marrow-derived cells have the unique ability to promote healing activities that can produce cytokines and growth factors that accelerate healing in damaged tissues. While we do not yet know if the implanted cells secrete trophic factors that promote differentiation of endogenous cells, there is the potential that a portion of the regenerated muscle layers are formed in response to trophic factors secreted from the implanted cells.

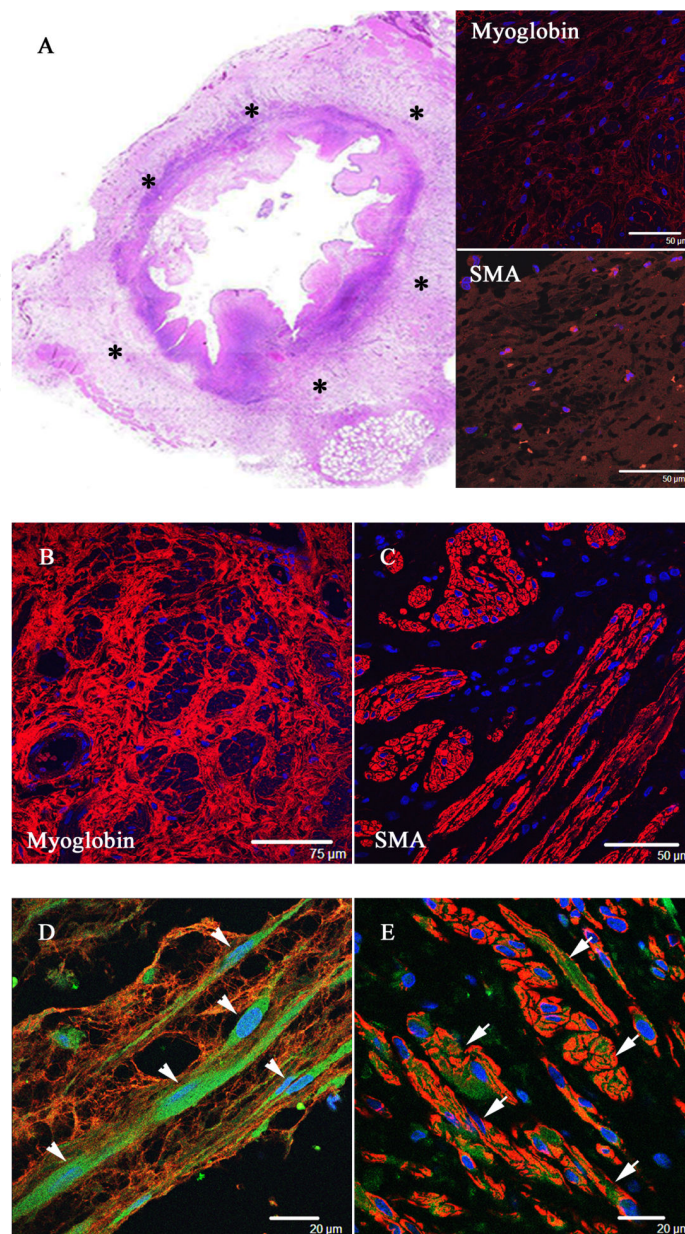
### **3.4. Differentiation of implanted bone marrow-derived cells**

At 7 and 14 days after implantation, we conduct double staining with GFP antibody in combination with striated muscle cell-, smooth muscle cell-, or myoblast-differentiation marker antibodies [2]. At 7 days, some of the implanted cells identified by the presence of antibody-labeled GFP are simultaneously positive for myoglobin or SMA antibody. These double positive cells show that the implanted autologous cells differentiate into striated or smooth muscle cells. These differentiated cells are widely distributed within the reconstructed muscle layers. At 14 days after implantation, the double-labeled cells appeared to form contacts among themselves, creating layered muscle structures (Figures 2D, E). In addition, the striated- and smooth-muscle differentiated cells contact non-GFP expressing muscle tissues that are presumably derived from the uninjured surrounding tissues. These cells are then integrated into the recovering muscle layers.

At 7 days after cell implantation, a few of the GFP-labeled implanted cells are simultaneously positive for Pax7, suggesting that they have myoblast properties [2]. In the development process to mature muscle, Pax7 acts as transcription factor, and satellite cells and myoblasts both express Pax7, but mature muscle cells do not [40]. Currently we cannot determine if the cells expressing both GFP and Pax7 are presumptive satellite cells or myoblasts. Nevertheless, the implanted cells clearly follow a development process that leads to the differentiation of striated or smooth muscle cells. The number of the cells expressing both GFP and Pax7 on day 14 is distinctly higher than on day 7 [2].

Myoblasts properly differentiate into striated or smooth muscle cells according to the surrounding environment. The greater number of Pax7 cells on day 14 compared to day 7 suggests that the formation rate of differentiated muscle cells may have decreased or even stopped. This suggests that the differentiation process of new striated and smooth muscle cell is under some type of intrinsic regulation. Understanding the controls for differentiation of the implanted cells is very important for further development of regenerative medicine. While the details of this regulation are currently unknown, it is clear that the presence of the myoblasts in the regenerated region may have important long term significance. In the event that the newly differentiated striated and/or smooth muscle tissues and structures spontaneously regress or are lost for other reasons, the presence of the myoblasts could ensure the replacement of the lost cells. Thus, the effects of treatments may be maintained for a long period of time.

We focus only on the implanted cells that maintained expression of GFP after implantation. At 7 days, the majority of both GFP and myoglobin, SMA, or Pax7 double-positive cells are



**Figure 2.** Implantation of autologous bone marrow-derived cells into freeze-injured urethral sphincters. (A) At 7 days after wounding, the urethral orifices are flaccid and gape open due to the loss of the surrounding muscle tissues (asterisks). The injured urethral sphincters lose the majority of the typical striated (upper right inset) and smooth muscle (bottom right inset) cells. Blue, nuclei. (B and C) The 14-day cell-implanted regions have distinct layered muscle structures containing numerous myoglobin- (B) and SMA- (C) positive cells that are similar to intact urethral sphincters. Blue, nuclei. (D and E) The 7-day cell-implanted regions have some GFP-positive cells that are simultaneously positive for myoglobin (D, arrowheads) or SMA (E, arrows) antibody. These cells are in contact with each other in the reconstructed muscle layers.

mononuclear. While we cannot definitively exclude the possibility of cellular fusion, the findings suggest that the number of these double-positive cells formed by cellular fusion is small. Thus, the GFP-labeled implanted cells differentiate into myoglobin-positive striated muscle cells and SMA-positive smooth muscle cells within the injured regions.

### 3.5. Recovery of leak point pressure

At 7 days after cell implantation, the leak point pressure of the cell-implantation group,  $13.15 \pm 2.82$  cmH<sub>2</sub>O, tends to be higher than the cell-free control group,  $8.13 \pm 2.43$  cmH<sub>2</sub>O, but the difference is not statistically significant. At 14 days, the leak point pressure of the cell-implantation group,  $17.82 \pm 1.31$  cmH<sub>2</sub>O, is significantly higher than that of the control group,  $11.78 \pm 3.23$  cmH<sub>2</sub>O ( $P < 0.05$ ) [2]. We do not yet know the leak point pressures of healthy rabbits, and whether or not the cell-implanted rabbits have voluntary control of the restored sphincters. Clinically, while less than 60–65 cmH<sub>2</sub>O of (abdominal) leak point pressure is one of the indexes of human stress urinary incontinence, it is not sufficient to diagnose it. Nevertheless, it is clear that increased or a high leak point pressure is helpful to inhibit urine leakage that can occur during physical activity. Therefore, cell therapy using bone marrow-derived cells has a great potential to reduce urinary incontinence and improve the quality of life.

## 4. Microenvironment

The microenvironment within the damaged recipient tissues affects regeneration of functional tissues [21]. We confirmed that bone marrow-derived cells implanted into uninjured normal tissue do not undergo differentiation and development. In injured tissues, bone marrow-derived cells exhibit significant potential to recover functional tissues. In addition, bone marrow-derived cells have the unique ability to differentiate into target cells and promote healing activities. However, these abilities are expressed only in suitable environments. In this section, we show that important roles are played in freeze-injured urinary bladders by the local microcirculation, large tissue pores, host tissue scaffolding, and expression of growth factor mRNAs that may support the differentiation.

### 4.1. Microcirculation in the freeze-injured urinary bladders

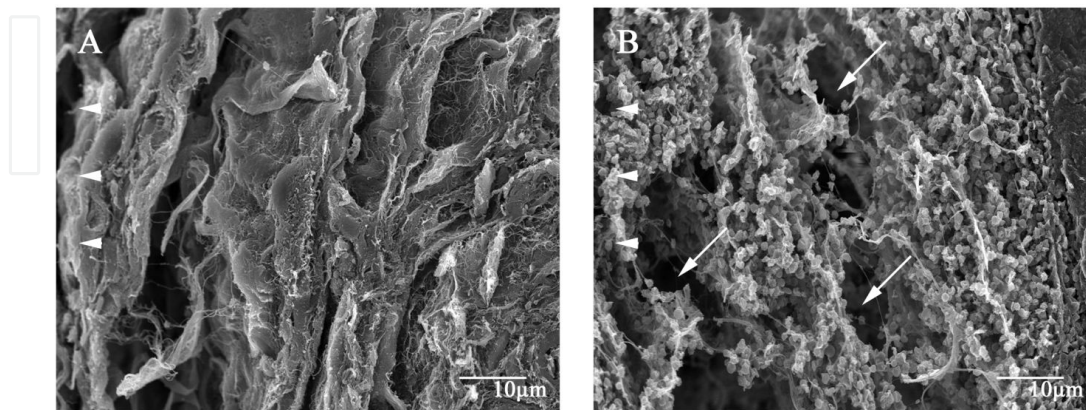
At 3 days after the freeze-injury of mouse urinary bladders, we observe the wounded areas by CCD video microscopy. Blood capillaries in the intact normal bladder walls have a robust flow of red blood cells with a velocity of  $0.26 \pm 0.03$  mm/s. In contrast, while maintaining a partial microcirculation, blood capillaries within the wounded bladder walls are not as abundant compared to normal bladder walls. Further, the blood flow velocity of the injured regions is  $0.12 \pm 0.11$  mm/s. The mechanism(s) for the reduced flow rate is not known with certainty. Regardless of the reason, the most important finding is that the injured regions are maintained with only a partial microcirculation [21]. The maintenance of at least a minimal microcirculation to provide oxygen and nutrition is likely to be one of the prerequisite factors necessary for successful tissue engineering.

### 4.2. Structures in the freeze-injured urinary bladders

We observe the intact normal and freeze-injured bladder walls by scanning electron microscopy. The normal bladder walls have smooth muscle cells organized into layers that are



readily apparent. These layers do not contain any porous spaces that are over 10  $\mu\text{m}$  in diameter (Figure 3A). In contrast, the freeze-injured bladder walls have few typical structures composed of smooth muscle cells, but there are many large porous spaces that are over 10  $\mu\text{m}$  in diameter (Figure 3B).



**Figure 3.** Layered smooth muscle structures within the freeze-injured urinary bladders. (A) The intact bladder walls contained layered structures composed of smooth muscle cells. (B) The freeze-injured regions had many large porous spaces (arrows). Arrowheads: exterior surface of the bladder wall.

By transmission electron microscopy, the normal bladder walls contain spindle-shaped smooth muscle cells with readily apparent nuclei. These cells are arranged in sheets and connected with each other by gap junctions. In contrast, smooth muscle cells in the freeze-injured bladder walls are shrunk, and exhibit blebbing [21]. The chromatin is condensed and nuclear fragmentation is apparent. Also, gap junctions are rarely present between the remaining cells of the smooth muscle layers. Based on the cytological observations, smooth muscle cell death is predominantly due to apoptosis, though we cannot exclude the occurrence of necrosis, especially immediately after the freezing injury.

The freeze-injured bladder walls contain numerous large pores, like those seen by a scanning electron microscopy, that are not present in the normal bladder walls [21]. The origin of these pores is not certain, but may be due to loss of smooth muscle cells that are the principal component of the wall in intact urinary bladders. In fact, the pores within the freeze-injured urinary bladders may be helpful in establishing a high rate of cell implantation and survival. They may also serve as scaffolding for the reconstruction of tissue structures.

#### 4.3. Expression of growth factor mRNAs in the freeze-injured urinary bladders

Using real-time RT-PCR arrays, we estimate that 84 growth factor mRNAs are expressed in the freeze-injured bladders [21]. Nineteen of these exhibit at least a two-fold increase over the intact normal bladders. The most impressive increases are for secreted phosphoprotein 1 (SPP1), inhibin beta-A (INHBA), glial cell line derived neurotrophic factor (GDNF), and transforming growth factor, beta 1 (TGFB1). TGFB1 specifically promotes differentiation of smooth muscle cells from bone marrow-derived cells [41-43]. The others, SPP1 [44,45], INHBA [46-48], and GDNF [49-51], also support differentiation of smooth muscle cells from bone marrow-derived

cells. Moreover, inflammation-related cytokine growth factor mRNAs for interleukins (IL)-6, -11, -1A, -1B, and -18 are upregulated along with angiogenic-associated growth factor mRNAs for epiregulin (EREG), chemokine (C-X-C motif) ligand 1 (CXCL1), teratocarcinoma-derived growth factor (TDGF1), fibroblast growth factor 5 (FGF5), C-fos induced growth factor (FIGF), and vascular endothelial growth factor A (VEGFA) that have the potential to improve microcirculation within the injured regions. In addition to the above growth factors, expression of trefoil factor 1 (TFF1), colony stimulating factor 3 (CSF3), hepatocyte growth factor (HGF), and bone morphogenetic protein 1 (BMP1) mRNAs is also elevated. The roles of these growth factors are unclear, but it is likely that they participate in wound healing.

Collectively, these results show that cells of the urinary bladder respond to freeze injury by enhanced transcription of mRNAs specifically associated with differentiation of smooth muscle cells and wound healing. If translated, expression of these genes can promote growth and development of a suitable physical and biochemical environment. Under these circumstances, the microenvironment within the freeze-injured urinary bladders would promote organization of the developing cells into physiologically functional tissues.

#### **4.4. Uninjured regions in the freeze-injured urinary bladders**

It is likely that recovery within the freeze-injured urinary bladders requires participation of the undamaged tissue adjacent to the injured site [1, 21]. In general, the success of implanted undifferentiated cells depends upon the recovery of host cells to provide an appropriate microenvironment at the location of the injury or disease site. These host cells are necessary to support the production of growth factors by the implanted bone marrow-derived cells [52-54]. The absence of a supportive microenvironment in the surrounding host tissues, as might occur in cases of irreversible or chronic diseases and/or injuries of the urinary bladder due to spinal injury or radiation therapy, might prevent or limit the recovery processes associated with the implanted cells.

#### **4.5. Tissue engineering**

Tissue engineering consists of three components: (1) undifferentiated cells having the potential to differentiate into specific cell types, (2) scaffolding to support construction of tissue structures, and (3) growth factors to promote differentiation of various and specific cell types. The bone marrow-derived cells are an excellent source of multipotent undifferentiated cells that can develop into smooth muscle cells [1, 14, 55, 56]. The tissue pores that are present three days after freeze-injury operation are likely to provide scaffolding and spaces suitable for colonization by the implanted bone marrow-derived cells. This would optimize the chance for a high rate of cell survival and differentiation [21]. Though we have not actually measured the secretion of growth factors by the surviving cells, at least 19 different growth factor mRNAs are increased three days after the freeze-injury operation. These mRNAs includes growth factor mRNAs for SPP1, INHBA, GDNF, and TGFB1 [21]. If they are translated, they would be able to support the differentiation of the implanted bone marrow-derived cells into smooth muscle cells. Finally, the maintenance of a minimal microcirculation within the injured regions probably supports growth and development of the implanted bone marrow-derived cells [21].



For all of these reasons, the freeze-injured urinary bladders provide a suitable microenvironment for differentiation and development of the implanted cells.

Recipient tissues do not always have a suitable microenvironment for the implanted cells. Thus, there is a need for new investigations that develop novel combinations of scaffolding and/or growth factors to support tissue engineering of stem-type cells that promote regeneration in severely damaged organs. In many cases, there might not be an adequate scaffold in vivo to support the implanted cells. Under those circumstances, it may be possible to construct scaffolds in vitro using biocompatible materials. To promote appropriate cellular differentiation, growth factors delivered by sustained-release or other drug delivery systems also may be necessary.

## 5. Conclusion

This chapter shows that bone marrow-derived cells have the potential to be an important cell source for regeneration of lower urinary tracts. The implantation of bone marrow-derived cells can produce functional smooth muscle layers in irreversibly damaged urinary bladders associated with the loss of smooth muscle layers due to injury or disease. Also, the cell implantation can recover functional urethral sphincters that prohibit the inadvertent release of urine. We suggest that to develop the full clinical potential of regenerative medicine, we need a further understanding of the requirements for undifferentiated cell proliferation and targeted differentiation. Moreover, based on tissue engineering principles, knowledge of each unique microenvironment within recipient tissues is necessary.

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