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Disc Regeneration Therapy Using Marrow Mesenchymal Cell Transplantation

A Report of Two Case Studies

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Study Design. Marrow mesenchymal cells (MSCs) contain stem cells and possess the ability to regenerate bone, cartilage, and fibrous tissues. Here, we applied this regenerative ability to intervertebral disc regeneration therapy in an attempt to develop a new spinal surgery technique.

Objective. We analyzed the regenerative restoration ability of autologous MSCs in the markedly degenerated intervertebral discs.

Summary of Background Data. Fusion for lumbar intervertebral disc instability improves lumbago. However, fused intervertebral discs lack the natural and physiologic functions of intervertebral discs. If intervertebral discs can be regenerated and repaired, then damage to adjacent intervertebral discs can be avoided. We verified the regenerative ability of MSCs by animal studies, and for the first time, performed therapeutic intervertebral disc regeneration therapy in patients and obtained favorable findings.

Methods. Subjects were 2 women aged 70 and 67 years; both patients had lumbago, leg pain, and numbness. Myelography and magnetic resonance imaging showed lumbar spinal canal stenosis, and radiograph confirmed the vacuum phenomenon with instability. From the ilium of each patient, marrow fluid was collected, and MSCs were cultured using the medium containing autogenous serum. In surgery, fenestration was performed on the stenosed spinal canal and then pieces of collagen sponge containing autologous MSCs were grafted percutaneously to degenerated intervertebral discs.

Results. At 2 years after surgery, radiograph and computed tomography showed improvements in the vacuum phenomenon in both patients. On T2-weighted magnetic resonance imaging, signal intensity of intervertebral discs with cell grafts was high, thus indicating high moisture contents. Roentgenkymography showed that lumbar disc instability improved. Symptom was alleviated in both patients.

Conclusion. The intervertebral disc regeneration therapy using MSC brought about favorable results in these 2 cases. It seems to be a promising minimally invasive treatment.

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Lumbar spine instability causes low back pain, and in intervertebral disc instability, dynamic damage to nerve roots induces lower extremity neurologic symptoms. In patients who do not respond to conservative therapy, spinal cord decompression and spinal fusion have been performed. Spinal fusion stabilizes the lumbar spine, thus improving low back pain, and because nerve roots are not dynamically damaged in fused intervertebral spaces, neurologic symptoms also improve.[1–4]

However, spinal fusion eliminates the natural and physiologic functions of intervertebral discs. Intervertebral discs connect the spine, contain nucleus pulposus, and are surrounded by anulus fibrosus. Nucleus pulposus is a highly elastic gel, and it absorbs shock applied to the spine. Spinal fusion places more stress on those discs adjacent to fused intervertebral discs, thus accelerating the regressive degeneration of adjacent discs. In the long term, prognosis for fusion can be poor.[5–8] Also, it has problems with damage because of autologous bone harvesting for fusion.[9–14]

The ideal treatment is to conserve the physiologic function of intervertebral discs and flexibility of the spine without spinal fusion. If it were possible to regenerate intervertebral discs, the prognosis for spinal surgery would improve markedly. Although research has been conducted on intervertebral disc regeneration using somatic stem cells,[15–23] to the best of our knowledge, there have been no reports on intervertebral disc regeneration therapy in clinical settings.

We previously reported that intervertebral disc-like tissue could be regenerated by grafting cultured marrow cells to damaged intervertebral discs, and that the tissue-regenerating ability of marrow cells could be applied to intervertebral disc regeneration.[20] About 3 years ago, we were the first group to perform intervertebral disc regeneration therapy using marrow cells in 2 patients, and favorable results were obtained.

Materials and Methods

The present disc regeneration therapy using marrow mesenchymal cells (MSCs) was submitted to the university's Ethics Review Board, was approved in June 2004, and was implemented in March 2005. The present therapy is the first of its kind to be performed anywhere in the world, and the following explanations were given to the patients and their families when obtain-

ing written informed consent: the objectives and methods of the present therapy; expected effects and risks; other possible treatments; refusal to consent to participate in this study would not result in any negative consequences; consent could be withdrawn at any point; and privacy would be properly protected. Therapy was performed after obtaining informed consent.

Subjects satisfied the following criteria: (1) magnetic resonance imaging (MRI) confirmed intervertebral disc degeneration; (2) radiograph showed the vacuum phenomenon; (3) roentgenkymography revealed intervertebral disc instability; (4) at the level of the degenerated intervertebral disc associated with the vacuum phenomenon and instability, pressure and spontaneous pain were seen, and when wearing a corset, low back pain was alleviated, and thus, the patients were diagnosed with symptomatic disc; and (5) complicated by lumbar spinal stenosis and neurologic symptoms, resisted conservative therapy, and required surgery.

Both the patients were women, and radiograph confirmed marked intervertebral disc regressive degeneration and the vacuum phenomenon. Patients had intervertebral instability, low back pain, and lower extremity neurologic symptoms, and they did not respond to conservative therapy. The vacuum phenomenon refers to intervertebral regressive degeneration and cavitation that appears as dark spots on radiographs. The clinical significance of tissue regeneration in intervertebral cavities is substantial. Intervertebral disc regeneration improves the vacuum phenomenon, facilitating assessment of postoperative intervertebral disc regeneration.

Marrow Mesenchymal Cell Culturing and Grafting

Preparation of Human Marrow Mesenchymal Cells. Approximately 5 mL of bone marrow fluid was aspirated from the ilium under local anesthesia. To prevent bone marrow fluid coagulation, 1 mL heparin was placed in a 10-mL injector beforehand (1000 U, Fuji Pharma Co., Japan) and was mixed with the bone marrow fluid. Bone marrow fluid was centrifuged (1000 rpm for 5 minutes), and after eliminating the supernatant and adding 5 mL physiologic saline, it was again centrifuged. The supernatant was aspirated, and the heparin was removed before incubation. Bone marrow fluid was placed in a T75 flask (Falcon) and placed in a carbon dioxide incubator (temperature, 37°C; humidity, 100%; and CO_2, 5%). Eagle minimum essential medium (Sigma Co., St. Louis, MO) containing 15% autologous serum, an antibiotic (gentamicin; Schering Plough), and 100 nmol/L estriol (Nacalai Tesque, Kyoto, Japan) was used.[24] Culture medium was exchanged 3 times per week. After 2 to 4 weeks (4 weeks in case 1 and 2 weeks in case 2), cultured cells were treated with 0.1% trypsin (Nacalai Tesque), and the resulting cells were centrifuged at 1000 rpm for 5 minutes. Centrifuged cells were rinsed once using physiologic saline, and a cell suspension of 10^5 cells/mL was prepared. Before surgery, culture medium was tested for bacteria, fungi, mycoplasma, and endotoxins to confirm the absence of microbial infection.

Cultured cells were placed in a 10-mL disposable injector, packaged aseptically, and brought to the operating room in an ice chest. About 20 pieces of collagen sponge were placed in the autologous MSC suspension for about 10 minutes to ensure cell penetration. In cell grafting, the instruments for percutaneous nucleus pulposus removal were used, and under radiograph guidance, a narrow tube was inserted into a target intervertebral space to graft pieces of collagen sponge containing autologous MSCs 1 at a time, and the cells were exactly grafted

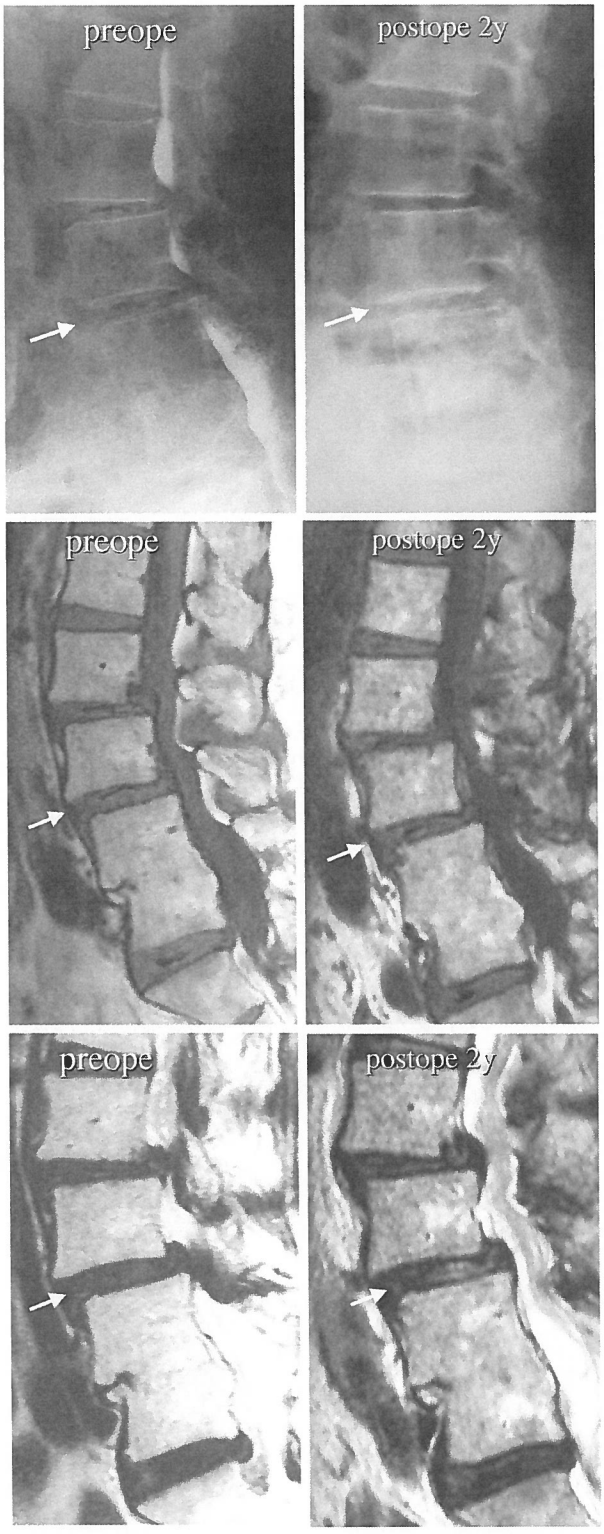

Figure 1. Marrow cells were cultured for 2 to 4 weeks. Pieces of collagen sponge were soaked in cultured mesenchymal cell solution and percutaneously grafted to intervertebral disc cavities under radiographic guidance.

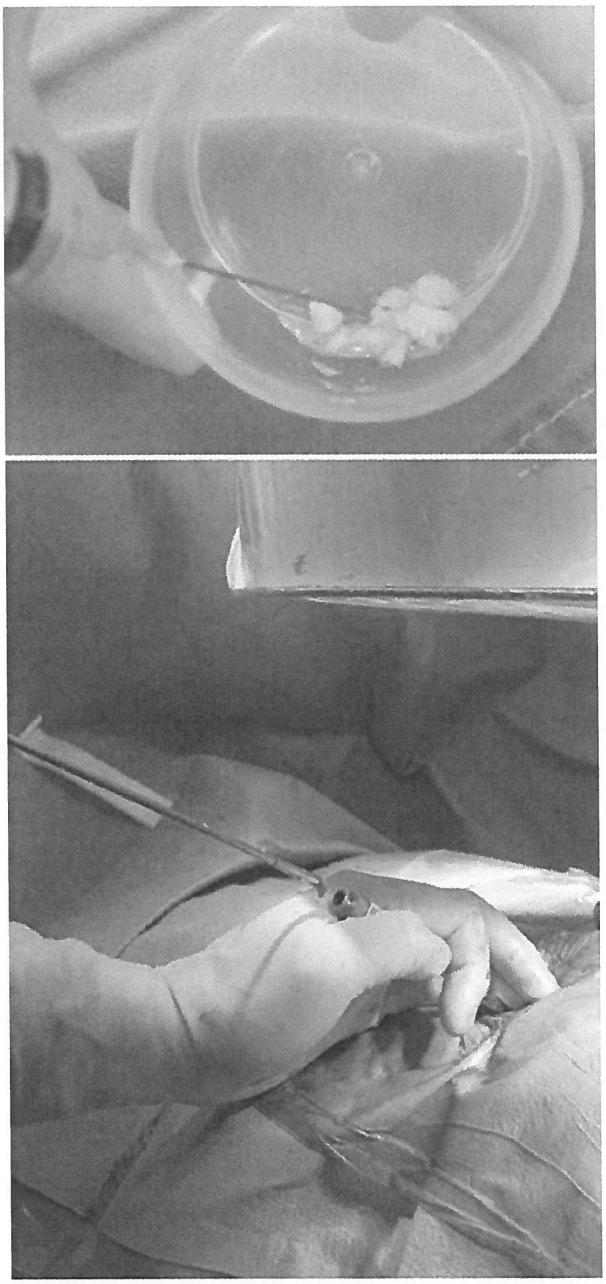

Figure 2. Radiograph and MRI images before (left column) and 2 years after (right column) surgery in case 1. A preoperative myelogram (upper left) showed lumbar spinal stenosis in L2–L3 and L3–L4 (arrow) and intervertebral vacuum phenomenon. A radiograph taken at 2 years after surgery showed that the intervertebral vacuum phenomenon in L2–L3 and L3–L4 (arrow) intervertebral disc had improved. T2-weighted MRI showed that the signal intensity in L2–L3 and L3–L4 (arrow) was low before surgery (lower left), but at 2 years after surgery, the signal intensity in L2–L3 and L3–L4 (arrow) was high, thus indicating increased moisture content.

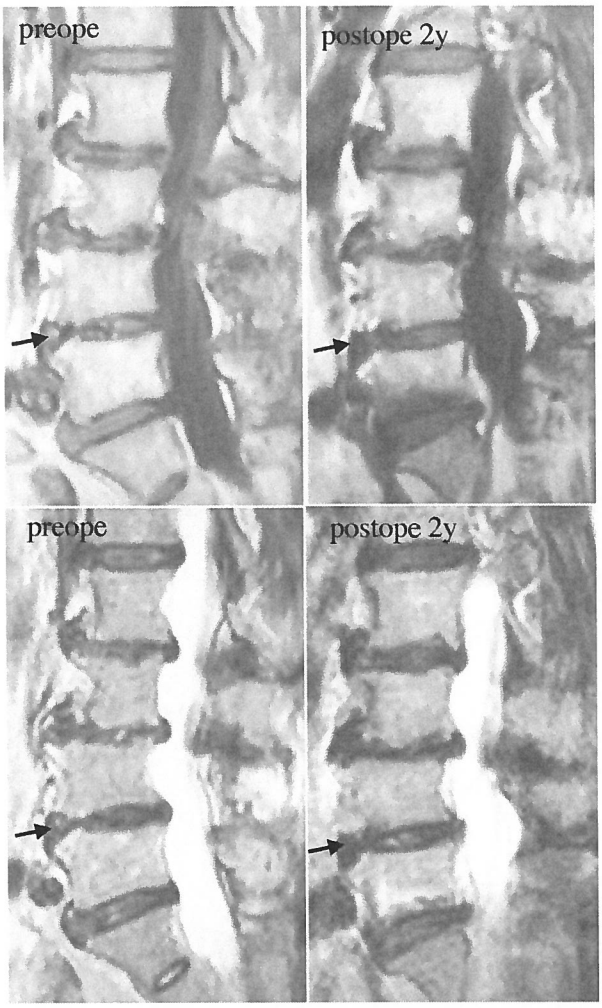

Figure 3. Preoperative myelography and computed tomography (CT) (left), radiograph taken 2 years after surgery (middle) and CT taken 2 years after surgery (right) in case 2. Preoperative myelography (left) confirmed L4–L5 vacuum phenomenon and lumbar spinal stenosis. Radiograph (middle) and CT (right) taken 2 years after surgery showed that L4–L5 vacuum phenomenon had improved.

into the central regions of discs as shown in Figure 1. After grafting of the cells, the hole was closed with collagen sponge (without cells).

The collagen sponge was prepared from the artificial dermis (Pelnac; Gunze Limited Co., Japan[25]) which consists of a 150-μm-thick silicone film and a 3-mm-thick collagen sponge with a gap size of 70 to 100 μm and porosity of 80% to 96%. The collagen sponge was cut into 3 mm × 3 mm squares, the silicone membrane was removed, and 1 mL of the above cell suspension was soaked onto it. About 20 pieces of the collagen sponge were grafted into the degenerated disc.

Clinical Course for Case 1

The patient was a 70-year-old woman having low back pain and right lower leg pain as chief complaints. About 15 years previously, the patient underwent anterior interbody fusion of L4–L5 because of left lower leg numbness and low back pain. At about 6 years after surgery, she began to experience low back pain and right lower leg numbness, and because her symptoms did not improve, she visited the hospital. Radiograph showed favorable anterior interbody fusion and spinal decompression in L4–L5, but intervertebral vacuum phenomenon, instability, and lumbar spinal stenosis were observed in L2–L3 and L3–L4 (left images in Figure 2). Because of anterior inter-

body fusion in L4–L5, we determined that fusion of the superior lumbar vertebrae would be inappropriate, and as a result, intervertebral disc regeneration therapy was recommended, and the patient consented.

Five milliliters of marrow fluid and 100 mL blood for autologous culture serum preparation were collected. Autologous MSCs were prepared as described earlier. After culturing for 4 weeks and confirming cell proliferation, fenestration was performed on L2–L3 and L3–L4. Pieces of collagen sponge were then soaked in the MSC solution, and under radiograph guidance, cells were grafted into the central regions of both discs percutaneously as shown in Figure 1.

Postoperative radiograph showed that intervertebral disc cavities were filled with collagen sponge pieces containing MSCs, and after surgery, vacuum phenomenon improved. For 2 weeks after surgery, the patient stayed in bed to allow the surgical wounds to heal, and using a flexible corset, standing and walking rehabilitation was initiated. Symptoms improved, and the patient was discharged at 1 month after surgery. The patient wore the flexible corset for 2 months after surgery.

At 6 months after surgery, low back pain disappeared, and left lower leg numbness and pain was alleviated. The patient then experienced mild low back pain, but at 2 years after surgery, the Visual Analog Scale score for low back pain has decreased to 38%, and symptoms have improved. Japanese Orthopaedic Association scores also improved from −3 to 9 points.

At 2 years after surgery, radiographs confirmed that the vacuum phenomenon in the intervertebral disc spaces has improved (Figure 2). Although no notable findings were seen on T1-weighted MRI, signal intensity of the intervertebral discs with cell grafts was higher on T2-weighted images, indicating high moisture content (lower images in Figure 2).

Clinical Course for Case 2

The patient was a 67-year-old woman. Because low back pain and leg pain persisted for several years, the patient was referred to the hospital to undergo surgery. Plain radiograph showed L4–L5 instability and vacuum phenomenon. Myelography showed lumbar spinal stenosis in L4–L5 (left image in Figure 3 and left image in Figure 4). Marrow fluid and blood for culture serum preparation were collected, and autologous mesenchymal cells were cultured as described earlier. Culture was performed for 2 weeks, and cell proliferation was confirmed.

After performing fenestration in L4–L5, as in case 1, pieces of collagen sponge containing autologous MSCs were grafted percutaneously in L4–L5 (Figure 1). After 2 weeks of bed rest after surgery, standing and walking rehabilitation was initiated using a flexible corset. Symptoms improved, and the patient was discharged at 1 month after surgery. The patient wore the flexible corset for 2 months after surgery.

At 2 years after surgery, radiograph and computed tomography confirmed that the intervertebral vacuum phenomenon improved (Figure 3). On T2-weighted MRI, the signal intensity of the intervertebral disc with cell grafts was higher when compared with that before surgery, indicating higher moisture content (lower image in Figure 4). After surgery, low back pain, lower leg numbness, and pain improved, and at years after surgery, Visual Analog Scale scores for symptoms decreased to 18%. Japanese Orthopaedic Association scores also improved from 8 to 25 points.

■ Discussion

Case 1 underwent anterior fusion of L4–L5 more than 10 years previously, and additional fusion would have been highly invasive and would have eliminated the physiologic function of the spine. In this patient, the intervertebral disc regeneration therapy that we developed was suitable for the treatment of intervertebral instability. The affected intervertebral space in case 2 also exhibited marked degeneration. Severe degeneration was also seen in adjacent intervertebral discs. With intervertebral disc regeneration therapy, the physiologic function of the lumbar spine was conserved, and low back pain and neurologic symptoms improved. Both the patients were very satisfied with the outcome. At present, research on intervertebral disc regeneration is at the stage of animal studies, but studies have been conducted on regenerating intervertebral discs using cytokines, gene therapy, autologous intervertebral disc cells, or stem cells.

Intervertebral disc degeneration involves cytokines and growth factors.[26,27] Growth factors, such as transforming growth factor-beta, epidermal growth factor, fibroblast growth factor, and bone morphogenetic protein-7, have been reported to exhibit intervertebral disc repairing effects.[28,29] Although therapy using growth factors is promising, further research on therapeutic effects is required. A study on intervertebral disc regener-

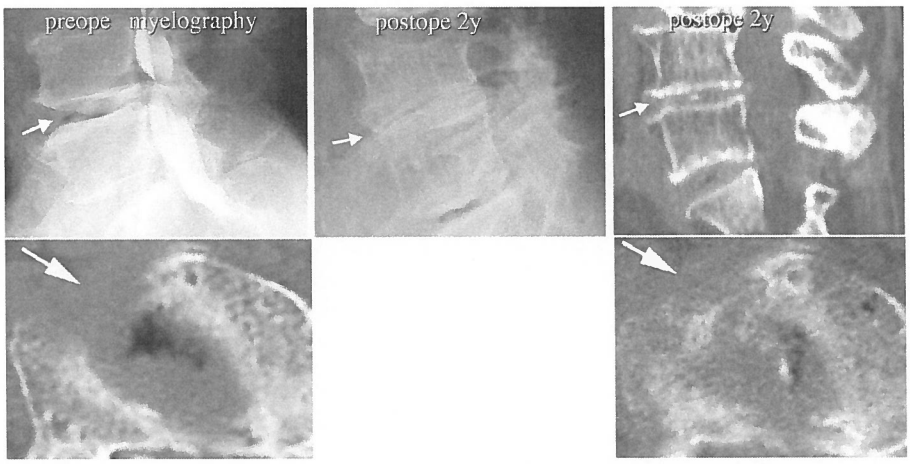

Figure 4. MRI images before (left) and after (right) surgery in case 2. On T2-weighted MRI (lower), the signal intensity of L4–L5 intervertebral disc was low (lower left), but at 2 years after surgery (lower right), the signal intensity of L4–L5 intervertebral disc was high, indicating increased moisture content.

ation therapy based on gene therapy was reported.[30–32] However, the side effects of gene therapy have not been fully clarified, and many ethical issues remain, and as a result, gene therapy is not yet practical.

With regard to research on intervertebral disc regeneration using stem cells, intervertebral disc regeneration using stem cells that are isolated and extracted from fat tissue seems to be promising.[33] However, a large amount of fat tissue is required for stem cell extraction, and further research is required for clinical application. In addition, specialized and expensive equipment is required for stem cell isolation and extraction.

Studies have been conducted on the use of differentiated intervertebral disc cells using dog models,[34,35] and there have been pilot clinical trials. Favorable clinical results were obtained by collecting intervertebral disc tissue and then isolating, incubating, and proliferating intervertebral disc cells.[36] To obtain intervertebral disc cells, it is necessary to damage healthy intervertebral tissue, collect tissue, and treat it with collagenase for long periods of time. Although differentiated intervertebral cells are practical, proliferation potential is low, and incubation and proliferation are time consuming.

On the other hand, with intervertebral disc regeneration therapy using MSCs, cells can be obtained by tapping, which is a minimally invasive procedure, and when compared with separating stem cells from fat tissue or cell separation from intervertebral disc tissue, healthy tissue is not damaged for cell collection. This is a convenient technique that does not require specialized equipment or complex procedures, such as long-term collagenase treatment. There have already been clinical reports on tissue regeneration therapy using MSCs,[37–43] and such techniques are practical.

After performing *in vitro* studies on intervertebral disc regeneration, Yamamoto *et al*[18] reported that nucleus pulposus cells were activated by coming into direct contact with MSCs. Risbud *et al*[19] reported that marrow mesenchymal stem cells differentiated into intervertebral disc cells. *In vivo*, Crevensten *et al*[17] grafted rat MSCs to the caudal vertebra and reported that even at 4 weeks after grafting, MSCs maintained viability and proliferated within the intervertebral disc. Sakai *et al*[16,23] conducted a study on rabbits and reported that grafting mesenchymal stem cells embedded in atelocollagen gel suppressed intervertebral disc degeneration from advancing. Cheung *et al*[21] conducted a study on rabbits and reported that grafting MSCs to intervertebral discs with advanced degeneration was very effective. These results indicate that grafting MSCs to degenerated intervertebral discs stimulates the metabolism of residual intervertebral disc cells, and as MSCs themselves differentiate into cartilage tissue, intervertebral disc-like tissue is regenerated.

We previously performed studies to document that marrow cells contain stem cells and possess the ability to regenerate various tissues, and investigated their regenerative abilities.[44–51] With approval of the university's Ethics Review Board, we have performed therapeutic regeneration therapy of various tissues in clinical settings.[39–43]

The present intervertebral disc regeneration therapy using MSC brought about favorable results in these 2 cases. The present therapy can be performed through an incision of about 5 mm, and seems to be a promising minimally invasive treatment. With future advances in culturing techniques and facilities in the field of regenerative medicine, intervertebral disc regeneration therapy will also advance.

■ Key Points

- If intervertebral discs can be regenerated and repaired, then damage to adjacent intervertebral discs associated with spinal fusion can be avoided, and the long-term prognosis for spinal surgery would improve.
- Research on intervertebral disc regeneration by somatic stem cells has been conducted; however, there have been no clinical reports of intervertebral disc regeneration therapy.
- We verified the regenerative ability of marrow mesenchymal cells by animal studies, and for the first time, performed therapeutic intervertebral disc regeneration in clinical setting.

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