Effects of implantation of bone marrow mesenchymal stem cells, disc distraction and combined therapy on reversing degeneration of the intervertebral disc

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Although success has been achieved with implantation of bone marrow mesenchymal stem cells (bMSCs) in degenerative discs, its full potential may not be achieved if the harsh environment of the degenerative disc remains. Axial distraction has been shown to increase hydration and nutrition. Combining both therapies may have a synergistic effect in reversing degenerative disc disease. In order to evaluate the effect of bMSC implantation, axial distraction and combination therapy in stimulating regeneration and retarding degeneration in degenerative discs, we first induced disc degeneration by axial loading in a rabbit model.

The rabbits in the intervention groups performed better with respect to disc height, morphological grading, histological scoring and average dead cell count. The groups with distraction performed better than those without on all criteria except the average dead cell count.

Our findings suggest that bMSC implantation and distraction stimulate regenerative changes in degenerative discs in a rabbit model.

Studies have shown that degeneration of the intervertebral disc produces progressive deprivation of oxygen and nutrients, leading to cell death and reduction of the matrix. Failure of delivery of nutrient is thought to be a main cause of disc degeneration, so it is unlikely that transplantation of cells into discs alone will affect the repair process.

Distraction may stimulate diffusion of water and nutrients into the intervertebral disc. Combining distraction of the disc and transplantation of bone marrow mesenchymal stem cells (bMSCs) may accelerate regeneration. There is no human or animal study that combines these therapies, and we therefore carried out a study in rabbits to assess this combined management.

Our aim was to determine the efficacy of transplantation of bMSCs, axial distraction and a combination of both treatments on retarding degeneration and stimulating regeneration of the disc.

Materials and Methods
This study was performed between December 2005 and October 2006 in the Animal Holding Unit of our institution after approval from the animal ethics committee, using skeletally mature New Zealand White rabbits.

The sample size required for bMSC implantation was calculated as follows:

$$n = \left( \frac{z_{1-\alpha/2} \sqrt{P(1-P)} + \sqrt{z_{1-\beta} P(1-P) + P(1-P) - P_1 + P_2}}{P_1 - P_2} \right)^2$$

where $n =$ sample size, $P_1 =$ anticipated proportion 1 (bMSCs treatment) = 0.9, $P_2 =$ anticipated proportion 2 (control group) = 0.1, $P = P_1 + P_2 = 0.5$, $Z_{\alpha/2} = 1.96$, and $Z_{\beta} = 0.842$.

The sample size required for axial distraction was calculated as follows:

$$n = \frac{1.96^2 \sqrt{(1-0.5) + 0.84 \sqrt{0.9(1-0.9) + 0.1(1-0.9)}}}{(0.9-0.1)}$$

where $n = 3$/group

$$n = \frac{1.96^2 \sqrt{(1-0.5) + 0.84 \sqrt{0.89(1-0.89) + 0.1(1-0.11)}}}{(0.89-0.11)}$$

where $n = 3.1/0.61 = 5.08$

$P_1 = 0.89$, $P_2 = 0.11$, and $P = 0.5$.

A larger sample size of five was chosen after suitable calculation. We assumed the drop-out rate to be 10% and so used six rabbits in each group.
The rabbits were randomly assigned into four groups that underwent different treatment regimens (Fig. 1): group 1 received bMCS transplantation alone; group 2 had axial distraction alone; group 3 received a combination of axial distraction and bMSCs transplantation; group 4 was a control group which only had loading/compression and a fixed unloading period.

The discs in all the rabbits were compressed using an external loading device with a compression force of 163.66 N, equivalent to five times the animal’s body-weight (3.4 kg) for two weeks. The compression device was adjusted daily to ensure the maintenance of a constant force throughout the study. The rabbits then had randomly assigned treatment regimens for eight weeks. The force of distraction, which was adjusted daily, was equivalent to five times the animal’s body-weight.

**Cell harvesting and culture.** Bone marrow stromal stem cells were aspirated from the iliac crest of five donor New Zealand White rabbits weighing between 2 kg and 3 kg three weeks before their transplantation. The bMSCs were isolated using centrifugal gradation density and a short-term adhesion on the tissue culture flask for culture. The cells were seeded into 75 cm² culture flasks in low-glucose Dulbecco’s modified Eagles medium (DMEM, Gibco, Green Island, New York) containing 10% fetal bovine serum (Gibco) and cultured at 37°C, in 20% oxygen and 5% CO₂. The stem cells were subcultured and harvested at passage 1. They were then embedded in atelocollagen (KOKENCELL-GEN; Koken Co. Ltd, Tokyo, Japan) solution until a final density of $1 \times 10^6$ cells/ml was attained; the 0.08 ml of bMSCs were then injected into the nucleus pulposus in rabbits from groups 1 and 3 by a posterolateral approach using a 25-gauge syringe guided by an image intensifier.
External spine compression and distraction device. The device (Fig. 2) could be used for both compression and distraction. It was used for compression by attaching a 40 mm long, compression spring (Fig. 2a) to the nut and the shaft. The spring had been calibrated and had a K value of 10.6 N/mm. The length of the compressed spring was measured by digital caliper. The device was used for axial distraction by changing the 40 mm spring for a 20 mm spring and attaching a spring holder to the nut (Fig. 2b). The spring had a K value of 9.6 N/mm. The length of the spring was monitored daily.

Surgical procedure. All the surgical procedures were performed by the same surgeons (HTH and HDI) using the same number and size of Kirschner (K)-wires. Under general anaesthesia the rabbits were shaved, cleaned and draped in a sterile fashion. A prophylactic dose (30 mg/kg) of cephalaxin was administered and the operation was performed through a dorsal approach to the lumbar spine. The external spine compression device, weighing 173.8 g, was attached using four stainless steel pins, 80 mm long and 5 mm in diameter, placed percutaneously and attached to two K-wires 7.5 mm in diameter inserted into the vertebral bodies of L4 and L5, parallel to the adjacent disc, by a variable-speed electric drill. A compression axial load of 163.66 N was applied to the disc two days after operation. The rabbits were allowed free unrestricted weight-bearing in their cages and were monitored daily.

In group 4, the external spine compression device was removed after two weeks of loading. In group 1 the device was removed after two weeks, and then 0.8 ml of bMSCs were injected via a posterolateral approach into the nucleus pulposus using a 25-gauge syringe guided by an image intensifier. In groups 2 and 3 the external compression device was changed to a distraction device as described above, and in group 3, bMSCs were transplanted into the discs.

All the rabbits were killed after two weeks of axial loading and eight weeks after bMSC transplantation in (group 1), axial distraction (group 2), injection of bMSCs and axial distraction (group 3), and axial unloading (group 4).

Tissue preparation. After removing the external device, the loaded intervertebral disc (L4-5) with adjacent vertebral bodies and the cranial adjacent segment (L3-4) were dissected out. Each dissected segment was placed in a bottle filled with saline solution. Radiographs and CT scans were carried out. The discs with their adjacent vertebral bodies from the four groups were placed in 4% paraformaldehyde and fixed overnight. After decalcification in 30% formic acid for up to 21 days, the discs were dehydrated in a graded series of ethanol before being embedded in paraffin. Sagittally orientated sections of 5 μm to 7 μm were placed on silane-coated slides. The sections were stained using haematoxylin and eosin (Sigma-Aldrich Corporation, St. Louis, Missouri) to demonstrate cell density and the general morphological structure. They were also stained with Alcian blue (Sigma-Aldrich) to assess proteoglycan grading. Two midsagittal sections of each disc were stained for dead cells using the TdT-dUTP terminal nick-end labelling (TUNEL) reaction (In Situ cell death detection kit, Boehringer-Ingelheim, Mannheim, Germany).

Disc height. Digital radiographs were taken using GE Senographe DMR+ with a senovision system to assess the lateral view of the discs. Both the loaded intervertebral disc (L4-5) with adjacent vertebral bodies and the adjacent cranial segment (L3-4) were scanned. The disc height was measured in our hospital's radiology centricity program.
(General Electric, Fairfield, Connecticut). On the lateral view, the height of the intervertebral disc was measured by calculating the average of the anterior, middle and posterior segments.

Micro CT scans were then taken to access the antero-posterior view of the discs. The thickness of the disc was measured by calculating the average of the right margin, left margin and middle of the frontal views. This was carried out by two of the authors (HTH and HDI), who were blinded to the groups. The average of the two readings was used in the study. The absolute differences in the height of the L4-5 disc were not analysed because of the variability in their heights. Instead, comparisons were made between the average of the heights of the L4-5 discs after intervention and the average heights of the L3-4 discs. The difference in heights between L4-5 and L3-4 was reported as a percentage of heights of the L3-4 disc.

Disc morphology and histology. The grade of disc degeneration was assessed macroscopically according to a five-category grading scheme developed by Thompson et al. This was modified into a four-grade classification in our study (Table I). The histology and architecture were analysed by standard light microscopy (Fig. 3) (20× Olympus, Tokyo, Japan). After preparation of the discs, the annulus fibrosus and nucleus pulposus were scored using a histological grading scale proposed by Masuda et al (12 points), and the vertebral end-plate was assessed according to a system developed by Boos et al (18 points) (Table II). The average histological scores in each group were calculated as below. A higher score would denote a more degenerate disc.

Table I. Morphological grading of intervertebral discs

<table>
<thead>
<tr>
<th>Grade</th>
<th>Nucleus</th>
<th>Annulus</th>
<th>End-plate</th>
<th>Vertebral body</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Bulging gel</td>
<td>Discrete fibrous lamellae</td>
<td>Hyaline, uniformly thick</td>
<td>Margins rounded</td>
</tr>
<tr>
<td>II</td>
<td>White fibrous tissue peripherally</td>
<td>Mucinous material between lamellae</td>
<td>Thickness irregular</td>
<td>Margins pointed</td>
</tr>
<tr>
<td>III</td>
<td>Consolidated fibrous tissue</td>
<td>Extensive mucinous infiltration; loss</td>
<td>Focal defects in cartilage</td>
<td>Early chondrocytes or osteophytes at margins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>of annular-nuclear demarcation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Horizontal clefts parallel to end-plate or Clefts extended through nucleus and annulus</td>
<td>Focal disruptions</td>
<td>Fibrocartilage extending from subchondral bone, irregularity and focal sclerosis in subchondral bone or diffuse sclerosis</td>
<td>Osteophytes &gt; 2 mm</td>
</tr>
</tbody>
</table>

The density of proteoglycan in the nucleus pulposus was assessed using a histological scale devised by Norcross et al in specimens stained using Alcian blue (Table III). A lower grade denotes a more severely degenerated disc, with grade 5 as the best and grade 1 the worst.

Dead cells (TUNEL reaction). Quantitative analysis was used to correlate the extent of cell death (TUNEL-positive cells) with the different treatment protocols. The number of cells that were positive using the TUNEL reaction was counted in each section with a fluorescent microscope (Olympus, Tokyo, Japan) with 200× magnification. The TUNEL-positive cells were bright green, and the unaffected cells appeared light red. The results were reported as the average of the total number of dead cells in the nucleus pulposus, annulus fibrosus and end-plate of the disc in the examined sections. The number of dead cells was counted by two of the authors (HDL and NCS) who were blinded to the groups. The average of the two counts was used in the analysis.

Carboxyfluorescein diacetate (CFDA) labelling of bMSCs. The bMSCs were labelled with CFDA, a non-fluorescent dye that turns fluorescent after cleaving by intracellular esterases. CFDA is inherited equally by daughter cells after division. The bMSCs were labelled using Vybrant CFDA SE Cell Tracer Kit (Invitrogen, Molecular Probes, Inc., Eugene, California). An additional four rabbits (two in group 1 and two in group 3) underwent transplantation with CFDA-labelled bMSCs. They were killed after week 4 and week 8, respectively.

Statistical analysis. The dependent outcome parameters assessed in the study were ‘disc height’, ‘morphological grading’, ‘histological scoring’, ‘proteoglycan grading’ and ‘cell viability’. The independent variable was ‘group’. Numerical data were analysed using the Kolmogorov-Smirnov test of normality. The disc height (according to the micro-CT scan), cell viability (according to the TUNEL reaction) and histological score were statistically analysed by a comparative analysis of variance (ANOVA) test. Analysis of disc height according to digital radiographs was by the non-parametric Kruskal-Wallis and Mann-Whitney tests. Morphological grading and proteoglycan grading were analysed by chi-squared tests.
L4/5 intervertebral discs with haematoxylin and eosin staining after intervention. a) Group 4 (control). The nucleus pulposus, annulus, and end-plate appear severely degenerated with more than 50% decrease in cellularity in the nucleus pulposus and moderately severe condensation of the extracellular matrix compared to L3-4. b) Group 1 (bMSC transplantation). Morphological structure and cellular density in nucleus pulposus, annulus fibrosus, and end-plate in L4-5 are relatively the same as in L3-4. c) Group 2 (distraction). Morphological structure and cellular density in nucleus pulposus, annulus fibrosus, and end-plate in L4-5 are relatively the same as in L3-4. d) Group 3 (bMSC transplantation and distraction). There is normal cellularity with large vacuoles in the matrix of the nucleus pulposus. Normal pattern of fibrocartilage lamellae (U-shaped in the posterior aspect and slightly convex in the anterior aspect) are seen in the annulus fibrosus.

Results
A total of 20 of 24 rabbits were included in the study protocol. Four, two from group 1 and two from group 3, died before the end of the study and were excluded. The mean weight of the rabbits increased from 3.34 kg (2.7 to 4.0) at the start of the study to 3.36 kg (2.7 to 4.4) by the fifth week, and to 3.44 kg (2.7 to 4.6) by the end of the study. **Disc height.** Digital radiographs and micro-CT scans (Fig. 4) demonstrated a 15.1% and 17.5% relative decrease in the height of the L4-5 disc compared to L3-4 in the control group (group 4). There were relative increases in the L4-5 disc height in group 1 (12.9%, 7.0%), group 2 (16.6%, 13.3%), and group 3 (16.7%, 10.8%) compared to L3-4. The differences in alteration in the disc height between group 4, the control group, and the other three groups achieved statistical significance (for digital radiographs between group 4 and 1, \( p = 0.011 \), group 4 and 2, \( p = 0.004 \) and group 4 and 3, \( p = 0.011 \); for micro-CT scans between group 4 and 1, group 4 and 2 and group 4 and 3 \( p < 0.001 \)). However, the differences in disc height alteration among
the three treatment groups did not reach statistical significance (between group 1 and 2 \(p = 0.522\), group 1 and 3 \(p = 0.831\) and group 2 and 3 \(p = 0.831\)).

**Morphological grading.** The discs from the rabbits in groups 2 and 3 were graded 1 in every specimen according to the modified Thompson criteria (Table I). The discs in group 1 were distributed equally in grades 1 and 3. Samples in the control group were distributed equally in grades 2, 3 and 4 (Fig. 5). A chi-squared analysis test showed that the morphological grading was significantly different between groups 2 and 4 \(p = 0.002\), as well as between groups 3 and 4 \(p = 0.019\).

**Histological score.** The rabbits in group 4 had the worst mean histological score of 68.8 (58 to 100). Those in group 3 achieved the best mean score of 30.6 (19.8 to 41). Groups 1 and 2 scored 39.6 (18.8 to 62) and 31.8 (16.7 to 49), respectively (Fig. 6). ANOVA testing with least significant difference post hoc analysis showed that there were signifi-
significant differences in the mean histological scores between groups 1 and 4 (p = 0.013), groups 2 and 4 (p = 0.001), and groups 3 and 4 (p = 0.010).

Proteoglycan grading. In group 1, 75% of the discs achieved a proteoglycan grading of 3 or above. In groups 2 and 3 all the discs had a grading of 3 or above (Fig. 7). All group 4 intervertebral discs obtained grade 3 proteoglycan grading (Fig. 8). Analysis showed that the proteoglycan grading was significantly different between groups 1 and 4 (p = 0.033), groups 2 and 4 (p = 0.002), and groups 3 and 4 (p = 0.005).

Cell viability. The mean total dead cell count was the lowest among group 2 cells (73.4 (60.5 to 116.4)), followed by group 1 (106.9 (63 to 181.8)) and group 3 (179.4 (112.3 to 222.8)). The highest dead cell count was found in group 4 (281.7 (159.5 to 397.5)) (Fig. 9). When analysing cell viability with respect to the site in the intervertebral disc, group 2 had the lowest number of dead cells in the annulus fibrosus (26.4 (22.5 to 31.5)) and the end-plate (19.0 (7.4 to 60.9)). Group 3 had the lowest average dead cell count in the nucleus pulposus (16.9 (6 to 24.3)). Group 4 had the highest number of dead cells in the annulus fibrosus (90.3 (46.3 to 96.3)) and the nucleus pulposus (104.9 (51.8 to 169.5)). Group 3 had the highest average dead cell count in the end-plate. There were significant differences in the average total dead cells between groups 1 and 4 (p < 0.0001), groups 2 and 4 (p < 0.0001), groups 2 and 3 (p = 0.010), and groups 3 and 4 (p = 0.012). Green fluorescence was observed to be evenly distributed in the discs of the four rabbits in group 1 and group 3 treated with CFDA-labelled bMSCs. After four weeks in vivo, the cell morphology exhibited a spindle-like shape but after eight weeks the nuclei of the cells appeared more rounded, with a denser extracellular matrix (Fig. 10).

Discussion

In a previous study, 9 using a model of inducing degenerative changes in intervertebral disc described by Kroeber et al., 10 we showed that axial compression loading of the intervertebral discs could lead to degenerative changes similar to those in a degenerative human intervertebral disc. We demonstrated that the rabbits which were loaded for two weeks showed deterioration in terms of disc height, morphology, MRI grading of disc degeneration, and cell viability. Rabbits between 20 and 24 weeks old were selected for the study to ensure they all had similar growth potential. Only rabbits below 24 months old were chosen, because there are no presumed degenerative changes in the intervertebral discs before then. 11 A total of four rabbits, two from group 1 and two from group 3, died before completion of the study. Although this reduced the sample size in these groups, statistical significance was still achieved for the comparison between the four groups.

Allogeneic bMSCs from donor rabbits were used for several reasons. The use of allogeneic instead of autologous bMSCs reduced physical stress on the rabbits used in the study. The use of allogeneic bMSCs will provide future potential commercial benefit in terms of availability of ready-to-use bMSCs. They will excite a relatively low immune reaction owing to the poor expression of HLA class II antigen. 12 The nucleus pulposus is relatively encapsulated and avascular, so that immunoreactivity will not occur. Atelocollagen, which contains 0.3% type II collagen, is used as a scaffold medium for cell culture because the intervertebral disc is made of collagen. Atelocollagen can facilitate the growth and development of the implanted cells in a three-dimensional environment, which is very suitable for disc cells. Sakai et al. 3 have shown success with the use of atelocollagen as a scaffold for bMSC transplantation in a rabbit model.

Sakai et al. 3 and Zhang et al. 13 have shown that bMSCs could survive for up to six months after transplantation into intervertebral discs in rabbits. In our study we have shown that bMSCs transplanted into degenerative discs survived for at least eight weeks. Transplantation of bMSCs into the degenerative discs in group 1 produced improvement in all the parameters observed compared to the control group (group 4). However, the improved morphological grading in samples of bMSCs in group 1 did not reach statistical significance compared to the control group. This might be because 0.08 ml of bMSC solution was used instead of the 0.02 ml used by Sakai et al. 3 The larger volume of bMSC solution used in our study possibly led to a greater degree of swelling of the nucleus pulposus, resulting in an increase in pressure on the periphery of the disc. This might have caused increased cell death and less favourable morpho-
logical findings. The second possibility is that we used a 25-gauge needle for transplantation instead of the 27-gauge needle with micro-injector used by Sakai et al. The slightly wider-bore might cause iatrogenic trauma to the disc, leading to degenerative changes. Sobajima et al. recommended stabbing the anterolateral annulus fibrosus of the rabbit intervertebral disc with a 15-gauge hypodermic needle to a limited depth of 5 mm to stimulate disc regeneration. We used a closed posterolateral approach, compared to Sakai et al., who used an open anterior approach. We applied these different methods and techniques because of their greater potential during clinical application. Whereas our study used compression to generate disc degeneration, Sakai et al. employed a nucleotomy method to induce degenerative disc disease which may not realistically simulate degeneration in the human intervertebral disc. Our use

of an image intensifier to guide posterolateral insertion of bMSCs has the potential for easy translation into clinical practice.

Despite the success in vivo of gene therapy,4 and bMSC transplantation in the rabbit model, the poor blood and nutrient supply, a low pH and the presence of inflammatory substances13 in the degenerative discs will be detrimental to the survival of the transplanted gene or bMSCs. Furthermore, stem cells differentiate along different cell lines, stimulated by the conditions of the host tissues. Thus the catabolic process of degeneration in such discs may lead to unpredictable differentiation of the transplanted stem cells. Hence, the physiological status of the discs should be restored before the application of molecular therapy to the disc.

One option to restore the physiological status of the affected disc is by distraction. A study by Kroeber et al13 suggested that recovery of the disc on a biological, cellular and biomechanical level could be induced by axial dynamic

Fig. 8a

Fig. 8b

Fig. 8c

Fig. 8d

a). Group 4 (control). Alcian blue staining of L4-5 intervertebral discs shows proteoglycans grade 3 staining. Large areas of faded alcian blue staining in the nucleus pulposus (black arrow) are identified. The staining in the annulus fibrosus (white arrow) appears patchy and inconsistent. b). Group 1 (bMSC transplantation). There is deep alcian blue staining in nucleus pulposus (black arrow) as well as deep staining between annulus fibrosus (white arrow) lamellae with gradual fading in periphery. c). Group 2 (distraction). Alcian blue staining of L4-5 intervertebral disc shows proteoglycan grade relatively similar to that of group 1. There are deep blue staining nucleus pulposus (black arrow) as well as annulus fibrosus (white arrow) d). Group 3 (bMSC transplantation and distraction). Alcian blue staining of the L4-5 intervertebral disc shows proteoglycan grade of 5. This is almost similar to those seen in the L3-4 intervertebral disc.
EFFECTS OF IMPLANTATION OF BMSCS DISC DISTRACTION ON REVERSING DEGENERATION OF THE INTERVERTEBRAL DISC

Bar chart showing cell viability recorded among the intervertebral discs in the study groups.

CFDA-labelled bMSCs in degenerated disc (200 × magnification). The cells survived and exhibited spindle-shaped morphology in a) group 1 and b) group 3 after four weeks. c) Group 1 and d) group 3 showed viable cells after 8 weeks. The cell nucleus appeared more rounded, with denser extracellular matrix.

distraction in the rabbit intervertebral disc. In a recent study, Guehring et al.\textsuperscript{16} showed that axial distraction stimulates regeneration in the degenerative discs by increasing expression of the extracellular matrix gene and the number of protein-expressing cells. Distraction also enhances hydration and nutrition\textsuperscript{2} in the degenerative discs, and axial distraction stabilised intradiscal pressure.\textsuperscript{17} Therefore, distraction not only stimulates disc regeneration, it also provides a favourable environment for bMSC transplantation.
In groups 2 and 3 the interventions achieved a significant increase in disc height with superior morphological and proteoglycan grading compared to the control group. Groups 2 and 3 also achieved superior results compared to the bMSC transplantation group (group 1). Axial distraction of the disc increased hydration and nutrition to the disc. Hydration increases hyalostatic pressure, which in turn stimulates expression of the matrix gene. Distraction can also increase expression of the extra cellular matrix gene, which will increase the capacity of the disc to absorb and retain water. These findings from other studies may explain the improvement in the height and proteoglycan content of the discs in these groups.

The rabbits in group 3 showed the best histological scores. Transplantation of bMSCs may lead to an increase in disc hydration, which in turn enhances repair of the extracellular matrix. Transplantation of bMSCs in atelocollagen may provide new cells to increase the cellular density in the nucleus pulposus, repair the annular disorientation and prevent a reduction in the level of proteoglycan. Distraction may reduce the dead cell count by providing a more optimal environment for the transplanted bMSCs by enhancing hydration and nutrition in the intervertebral disc.

The average dead cell count in groups 2 and 3 was significantly lower than in the control group. Guelhoing et al found that, in compression with compression, distraction increased gene expression, which regulated collagen type I, II, biglycan, and decorin. At the same time, bone morphogenetic protein (BMP-2), tissue inhibitor of matrix metalloproteinase (MMP-1) and fibromodulin levels registered a decline. The decrease in matrix-degradation enzymes such as MMP-1 will cause the pathways of apoptosis to decrease, which may account for the lower dead cell count in groups 2 and 3.

This is the first study to assess the effect of combining distraction and implantation of bMSCs in the reversal of disc degeneration. The combined intervention group produced the second highest average dead cell count based on the TUNEL reaction, and the highest dead cell count in the end-plate. However, the dead cell count was still significantly less than that in the control group (179.4 vs 281.7).

Axial distraction may also increase the pressure on the end-plate. We performed distraction and transplantation of bMSCs simultaneously. In studies focusing on the effect of distraction on reversing disc degeneration, the benefits of distraction were shown only after 28 days. Thus, by combining the two interventions simultaneously, sufficient time may be given for distraction to reverse the harsh environment of the distracted disc before the bMSCs are transplanted. This may account for the increased dead cell count at the end-plates noted in the combined intervention group. However, this approach may restore intervertebral discs toward regeneration in terms of the increase in height and the histological score, although our results did not achieve a statistically significant difference compared to bMSC transplantation or distraction alone. Further studies are needed to measure the pressure and volume in the nucleus pulposus after transplantation of bMSCs and axial distraction, and to determine the optimal timing for the transplantation of bMSCs into the nucleus pulposus after the intervertebral disc has been distracted.

This study confirmed that bMSCs and axial distraction can individually stimulate regeneration of the intervertebral disc. However, further studies are needed to investigate the interval between distraction and transplantation of bMSCs in order to achieve optimal results in reversing degeneration of the intervertebral disc.

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