Intradiscal Injection of Autologous Discogenic Cells in Patients with Discectomy: A Prospective Clinical Study of Its Safety and Feasibility

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Abstract

The treatment of intervertebral disc degeneration (IVDD) is still a huge challenge for clinical updated surgical techniques and basic strategies of intervertebral disc regeneration. Few studies have ever tried to combine surgery and cell therapy to bridge the gap between clinical and basic research. A prospective clinical study with a 72-month follow-up was conducted to assess the safety and feasibility of autologous discogenic cells transplantation combined with discectomy in the treatment of lumbar disc herniation (LDH) and to evaluate the regenerative ability of discogenic cells in IVDD. Forty patients with LDH who were scheduled to have discectomy enrolled in our study and were divided into the observed group (transplantation of autologous discogenic cells after discectomy) and control group (only-discectomy). Serial MRI and X-ray were used to evaluate the degenerative extent of index discs, and clinical scores were used to determine the symptomatic improvement. No adverse events were observed in the observed group, and seven patients in the control group underwent revisions. Both groups had significant improvement of all functional scores post-operatively, with the observed group improving more considerably at 36-month and 72-month follow-up. The height and water content of discs in both groups decreased significantly since 36 months post-op with the control group decreased more obviously. Discectomy combined with autologous discogenic cells transplantation is safe and feasible in the treatment of LDH. Radiological analysis demonstrated that discogenic cells transplantation could slow down the further degeneration of index discs and decrease the complications of discectomy.

Key words: intervertebral disc degeneration; cell transplantation; discogenic cells; discectomy; lumbar disc herniation.

Graphical Abstract

Autologous discogenic cells transplantation could potentially compensate for the shortcomings of discectomy by retarding the intervertebral disc degeneration.

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Introduction

Intervertebral disc degeneration (IVDD), a normal, unidirectional, and irreversible process with aging, is a predominant source of low back pain and has a significant socioeconomic impact given the associated disability. Low back pain is the most common clinical manifestation in the process of IVDD and is now the leading cause of disability globally. Lumbar disc herniation (LDH), commonly considered an extension of progressive disc degeneration, frequently comes with sciatica, numbness, and claudication in addition to low back pain. Intervertebral discs are the root or culprit of all these health problems.

The intervertebral disc is a special anatomical structure located between 2 vertebrae and accounts for one-third of the total human spine length, absorbing and distributing complex loads, and providing spinal stability while permitting motion. Macroscopically, the intervertebral disc is a special complex composed of a gel-like core-nucleus pulposus (NP), which is laterally encapsulated in layers of annulus fibrosus (AF) and sandwiched by 2 thin layers of cartilage endplate (CEP). Microscopically, these tissues consist of different but interrelated cell populations and specific matrix structures.

Despite the morphological differences in the structural organization of the different anatomical components of the IVD to which they belong, NP cells, AF cells, and CEP cells are closely interdependent and play a crucial role in maintaining the integrity and biomechanical function of the intervertebral disc. However, in the process of disc degeneration, degenerative changes in the biomechanical and structural properties of these 3 kinds of cells usually occur concurrently, resulting in imbalanced homeostasis, decreased cells and extracellular matrix (ECM), reduced intradiscal pressure, and reduced nutritional supply. All these domains consist of a vicious circle of disc degeneration, potentially inducing disarrangements such as intervertebral disc herniation, spondylolisthesis, and spinal stenosis.

LDH is the most frequent type of disc degeneration and the most common cause of radicular or sciatic pain. However, approximately 60%-90% of patients with LDH are mild and generally managed with conservative interventions, eg, physical therapy, medication, or block therapy. For the remaining patients at a severe stage or with refractory to conservative treatments for at least 6 months, surgical intervention should be considered. With the continuous development and progress of surgical technology, there have been many surgical techniques for LDH, ranging from discectomy to lumbar fusion to total disc replacement (TDR) and minimally invasive surgery under endoscopes. Surgical techniques are very effective for temporary neurological decompression by removing herniated intervertebral discs and partly eliminating chemical stimulation simultaneously in patients suffering from LDH. However, these surgical interventions remain highly controversial for their long-term efficacy and complications. Discectomy removes the disc tissue that compresses the nerve but destroys the integrity of the annulus fibrosus, resulting in 5.2% reherniation and 37.5% recurrence of sciatica. Lumbar fusion could remove the disc tissue completely at the index level with the sacrifice of segmental motion, leading to 36% postoperative complications, 27% revision, and 82.6% adjacent segment degeneration. Although TDR can partially preserve segmental mobility, the clinical effect is equivalent to that of fusion surgery, and the incidence of symptomatic adjacent disease after surgery is also as high as 34%. Minimally invasive surgery relieves the pain of patients with minimal trauma, but the incidence of disc reherniation is even higher than that of discectomy, and there is a higher risk of incomplete decompression. The root cause of all these consequences is that these surgeries do not intend to restore the IVD but to accelerate the degeneration process.

Against this backdrop, advancements in regenerative medicine have led to an effervescence of growth in the development of various experimental and preclinical trials aiming at restoring or re-establishing a healthy IVD. The 3 main methods are cell therapy, growth factor therapy, and gene therapy. Cell therapy is currently the most studied and has made major breakthroughs and successes in animal experiments and preclinical experiments and has gradually advanced from the lab to the clinic. It will likely fill the gap between conservative therapy and surgeries for the treatment of IVDD.

However, current clinical studies focus primarily on the safety and short-term efficacy of different types of cells and are still within the pilot or phase I stage without control groups. Moreover, most of these studies choose moderate disc degeneration as the indications for which it would be improved by conservative treatments. Although cell therapy represents a potentially low-risk and low-cost solution to address the tremendous unmet need for new treatment options for patients with disc degeneration, it is disadvantageous in treating
symptomatic LDH, especially in neural decompression. Cell therapy has not yet been clinically studied for disc repair after discectomy and for preventing accelerated disc degeneration. Although these treatments have used a variety of cell types, including autologous or allogeneic cells obtained from notochordal,16 chondrocyte-like cells,17 and mesenchymal stromal cells (MSCs),18 only a monotype of cell is used to repair the intervertebral disc, which is a complex composed of 3 kinds of cells in clinical research. Theoretically, these cells, which were transplanted into the discs, possess multiple proliferative capacities, but the hostile microenvironment in the degenerated IVD hallmark by low cellularity, hypoxia, acidic conditions, and mechanical loading remains the greatest challenge for transplanted cells to remain viable for a long time, not to mention for functioning well. There is still a long way to go to overcome these difficult problems, although the following solutions have been attempted: preconditioning the cells under the mimicking milieu of discs,19 activating the cells by cell-to-cell coculture,20 and combining them with growth factors or scaffolds.21,22 Autologous IVD-derived cell transplantation seems to be more natural physiologically and can avoid the risk of graft-versus-host reactions and ethical issues. More importantly, IVD-derived cells would be more capable of adapting to the harsh microenvironment and remain viable longer. This method dates back to 1998, and Nishmura and Mochida first reported that percutaneous reinsertion of autologous normal NP tissue could delay disc degeneration in a rat model.23 In 2002, the EuroDISC trial was initiated by Meisel and his colleagues.24-26 In their interim analysis, they found that autologous disc chondrocyte transplantation (ADCT) was technically feasible and biologically relevant to repairing disc damage and retarding disc degeneration. Later, Tschugg et al also gave their short report of the ongoing ANVOVART disc plus a study to assess the safety and efficacy of ADCT plus a hydrogel in the repair of herniated nucleotomized discs, but no specific results have been reported yet. Overall, although these studies used ADCT, the cells they used were actually only NP cells, as in the study of Mochida et al,20 which may influence the interaction of the 3 kinds of disc cells in the regeneration process and in the repair of the whole disc.

Taken together, we hypothesize that discogenic cells, ie, IVD-derived cells consisting of NP cells, AF cells, and CEP cells, would be the optimal kinds of cells to repair the disc. The optimal resource of discogenic cells is undoubtedly the disc tissues removed from discectomy in the treatment of disc herniation. These disc materials can be part of the NP, annulus fibrosus, endplate material, or a combination of the above. Moreover, our previous studies29,30 and other studies31-34 have demonstrated the presence of progenitor cells in the tissue of the IVD and compared the properties of IVD progenitor cells with MSCs. Lyu et al reviewed these studies and pointed out that IVD progenitor cells might have superior potential to non-IVD-derived MSCs for IVD cell differentiation and be better able to adapt to and engraft into host IVD tissue after transplantation. Together, transplantation of discogenic cells, which may consist of 3 kinds of cells and their progenitor cells, would potentially restore and repair the disc complex as a whole.

For patients with symptomatic disc herniation scheduled for discectomy, culturing the discogenic cells from the removed disc tissue and reinserter them into the index levels would take full advantage of both surgery and cell therapy, including symptom relief and prevention of accelerating degeneration simultaneously. However, no clinical study hitherto has assessed the long-term efficacy of this combined strategy.

In this study, we evaluated the safety and feasibility of discectomy followed by autologous discogenic cell transplantation in the treatment of LDH and the regenerative restoration ability of discogenic cells in clinical outcomes with long-term follow-up.

Materials and Methods

Study Design

This study was a prospective open-label, nonrandomized controlled clinical trial performed at a single center. Prior to undertaking the study, ethical clearance was obtained from the Ethics Committee of the Sixth Medical Center of PLA General Hospital (Beijing, China). All patients received an explanation of the project and signed the approved informed consent before the intervention. Each patient participating in the clinical trial underwent surgical treatment for their disc prolapse, and according to the patients’ willingness, those included in the observed group would accept the subsequent cell transplantation. The remaining patients who underwent only discectomy were classified as the control group. The flow-chart of our study is shown in Fig. 1.

Study Population

Patient Selection

We included participants meeting the following inclusion criteria (Table 1) who had been diagnosed with disc herniation and confirmed clinically and radiologically and who were scheduled for lumbar discectomy between May 2013 and September 2015 and then divided into the observed group (discectomy combined with cell transplantation) and the control group (discectomy alone) according to their choices.

Preoperative images of both groups are shown in Fig. 2. Patient demographic information, such as age, sex, and body

Figure 1. The flow chart of the study design.
mass index (BMI), was recorded after enrollment, and all the participants signed confidentiality agreements.

**Surgical Procedure**

All patients were placed in a prone position after general anesthesia. The level of the discectomy was verified using intraoperative fluoroscopy. A posterior middle-line approach was used, and back muscles were detached from the spinous process and retracted unilaterally. Fenestration was used as the caudal part of the cephalad lamina, and the ligamentum flavum was removed to expose the compressed nerve roots and herniated disc. Only herniated disc material and a small portion of the intervertebral disc were removed. The disc tissues in the control group were abandoned as usual, but the tissues in the observed group were collected and sent to the GMP lab for cell culture.

**Cell Production and Preparation**

Discogenic cells were isolated as described in our previous studies.\(^29,30\) Briefly, disc tissues were harvested during discectomy and transferred to the GMP lab for culturing. Disc tissues were washed 3 times with PBS. Vessels and ligaments surrounding the disc were removed as much as possible, but AF, NP, and CEP were retained, and disc samples were fragmented into no larger than 1-mm\(^3\) pieces with ophthalmic scissors. Subsequently, tissue fragments were digested with 0.2% (m/v) collagenase II (Sigma-Aldrich; Merck KGaG, Darmstadt, Germany) for 6 hours at 37 °C. Primary cells were obtained after filtering through cell strainers with a pore size of 70 µm, suspended in Dulbecco’s modified Eagle’s medium-low glucose supplemented with 10% fetal bovine serum (both from Sigma-Aldrich; Merck KGaG), and then seeded in 25-cm\(^2\) flasks at a density of 1 × 10\(^5\)/mL. Cells were cultured in an incubator at 37 °C in a humidified atmosphere containing 5% CO\(_2\) and passaged upon reaching 80% confluence. The medium was changed every 3 days, and discogenic cells at passage 3 were resuspended in Dulbecco’s modified Eagle’s medium at a density of 2 × 10\(^4\)/cells/mL. Before transplantation, the cells were harvested, washed twice, and resuspended in physiologic saline at a target volume for transplantation. The supernatant media were sent to the Department of Laboratory to test for bacterial, fungal, mycoplasma, and endotoxin contamination. Cultured cells were placed in a 5-mL disposable syringe, packaged aseptically, and brought to the operating room in an ice chest.

**Cell Transplantation**

Cell transplantation was conducted 4-5 weeks after discectomy to ensure that the annulus had healed and contained the cells. Once discogenic cells had been subcultured and suspended in normal saline, they were prepared for reinjection in a syringe. Patients were brought into the operation room and placed in a prone position on an operating room table, and after standardized sterile preparation, the injection site was treated with local anesthetic (1% buffered lidocaine). Using a pressure-volume test prior to the delivery of cells, cells could be placed with confidence that they would be
Discogenic cells were percutaneously injected into the index disc on the opposite side from the discectomy through a standard posterior lateral discogram approach over the superior articular process, with the starting point being as lateral as possible to allow as much of the injectate as possible to be injected into the posterior disc annulus with a 22-gauge needle under fluoroscopic guidance. Approximately 1-3 mL (4-7) × 10^6 of the cultured cells were injected into the symptomatic lumbar disc. The needle remained in place for 3-5 minutes after injection to prevent cell leakage. Patients were prescribed pain medicine to be used as needed for 1-7 days and placed on restrictions as tolerated. Physical therapy postinjection was not restricted but was encouraged.

Follow-Up Protocol
All patients were discharged postdiscectomy between the seventh and 12th days. Clinical evaluation and routine analysis were conducted for all the patients, and the observed group of patients needed extra cell transplantation. At 3, 6, 12, 36, and 72 months after discectomy, the patients were scheduled for outpatient clinic appointments via phone and/or email. They were clinically examined during their follow-up and followed with radiological imaging. Any participants who underwent a secondary fusion operation at any time terminated the later follow-up.

Clinical Assessment
The safety assessments mainly contained adverse events (AEs), disc herniation and revision related to the intervention during treatment and follow-up. The clinical efficacy involves the following scoring systems: the visual analog scale (VAS) was used to assess changes in leg and low back pain and the Oswestry Disability Index (ODI) was used to assess the limitations of various activities of daily living. The Short Form Health Survey-36 (SF-36) questionnaire and Japanese Orthopedic Association (JOA) scoring system for low back condition of the index discs.

Radiological Assessment
Radiological examinations were used to evaluate the regenerative ability of discogenic cells indirectly, including X-ray to measure the disc height and MRI to evaluate the hydration condition of the index discs.

Disc height: IVD height and vertebral height were converted to the disc height index (DHI) according to the methods applied by our previously published protocols. In short, the intervertebral DHI was obtained by calculating the average values from a medial plane at 3 points of the posterior, middle, and anterior parts of the involved IVD, and these values were divided by the average height of the neighboring vertebrae. DHI was determined as 2 × ((b1 + b2 + b3)/ (a1 + a2 + a3 + c1 + c2 + c3)) × 100% (Fig. 3A).

Water content of discs: 3.0T MRI (Philips, Medrad Spectris Solaris EP, USA) was performed to obtain midline T2-weighted sagittal images of index discs. The severity of IVDD at each follow-up was assessed using Pfirrmann’s classification. We used Photoshop version 2020 (Adobe, San Jose, CA, USA) to measure the grayscale of discs and cerebrospinal fluid at the same level. The grayscale of the disc was normalized against that of the cerebrospinal fluid and represented as the ratio value of the grayscale (RVG) (Fig. 3B). At the last follow-up, apparent diffusion coefficient (ADC) mapping from the diffusion-weighted coefficient (DWI) was performed on 6 patients in each group to quantitatively determine changes in water content in the index discs compared with that of normal adjacent discs. DWI data measurement was performed on an imaging workstation (Philips Extended MR WorkStation). First, we selected an appropriate region of interest (ROI) to measure the ADC value of the affected disc according to its size. Then, the same ROI was used to measure the ADC value of adjacent normal segments. Finally, the 2 values were used to normalize the ADC value of the index level (Fig. 3C). We also conducted ultrashort time-to-echo (UTE) MRI in these patients to evaluate the integrity of the CEP of index discs. CEP defects were defined as discontinuity of the high signal on UTE MRI (black arrow) on the cranial side and discontinuity of the high signal of CEP defect (white arrow) on the caudal side.

Statistical Analysis
The measurement data are presented as the means ± SD, and the counting data are expressed as percentages (%). Pearson’s chi-square, Fisher’s exact test, Student’s t test, the Mann-Whitney U test, or Wilcoxon signed-rank test was used to analyze the within- and between-group differences at baseline and post-treatment of each group for the clinical scores and radiological measurements. Repeated-measures analysis of variance...
Table 2. MR scanning parameters.

| Sequence | TR (ms) | TE (ms) | Slice (mm) | Flip angle (degree) | FOV (mm) | AP(freq.)*FH(phase) | Recon matrix | Gap (mm) | NSA |
|----------|---------|---------|------------|---------------------|----------|---------------------|--------------|----------|-----|
| T2WI     | 3000    | 120     | 4          | 90                  | 250      | 200 × 200           | 252 × 191    | 0.4      | 2   |
| DWI      | 3000    | 106     | 8          | 90                  | 96       | 96 × 96             | 76 × 73      | 8        | 1   |
| UTE      | 4       | 0       | 4          | 15                  | 200      | 200 × 200           | 200 × 200    | −2       | 1   |

T2WI, T2-weighted imaging; DWI, diffusion-weighted images; UTE, ultrashort time to echo; TR, repetition time; TE, echodeay time; FOV, field of view; AP, anterior and posterior; FH, foot and head; NSA, number of signal average.

was used to compare the significant differences between the groups at each time point. Patients without baseline scores were excluded from the respective analyses. Missing data (due to incomplete patient interviews, study withdrawal) were filled with a multiple imputation method to enable individuals with incomplete data to be included in the analysis. All data processing was performed utilizing IBM SPSS Statistics 26.0.

Results

Demographics

Forty patients met the inclusion criteria and were followed in the treatment registry. Out of 19 patients in the observed group, one subject refused to accept cell transplantation after discectomy and was included in the control group.

In the observed group (n = 18), 16 (88.9%) patients were males, and 2 (11.1%) were females, with a mean age at surgery of 36.39 years (SD = 11.93; min = 22; max = 59). BMI was averaged at 24.68 kg/m². Their duration of symptoms ranged from 6 to 28 months. All the patients were treated with a single-level discogenic cell transplantation following discectomy and was included in the control group.

In the control group (n = 22), 16 (72.7%) patients were males, and 6 (27.3%) were females, with a mean age at surgery of 40.73 years (SD = 12.01; min = 21; max = 59). BMI was averaged at 24.79 kg/m². Their duration of symptoms ranged from 7 to 33 months. All the patients were treated with single-level discectomy, with the L5-S1 level treated most commonly (10/18; 55.56%), followed by the L4-L5 IVD (8/18; 44.44%). The mean cell viability was 93.23%.

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Clinical Outcomes

Safety Variables

In the observed group, no patients suffered from immunoreactions, such as fever, allergic reaction, or local infection, from immediately after cell transplantation to 3 months postoperation. Two subjects (11.11%) experienced low back pain at 1 to 2 years postoperation, which was relieved by medication. One patient (5.56%) developed disc reherniation 72 months after the operation but refused to undergo secondary surgery. In the control group, 9 patients (36.36%) had low back pain and sciatica during the follow-up, of which 7 patients (31.82%) progressed to revision during the follow-up due to the following reasons: 4 patients (18.18%) elected to have surgery because of disc reherniation within 36 months postoperatively (11, 21, 28, and 35 months), 2 patients (9.09%) elected to have surgery because of lumbar canal stenosis of the index level between 48 and 72 months (50 and 68 months), and one patient (4.55%) elected to have surgery because of spondylolisthesis 56 months postoperatively. The total number of revisions (n = 0) in the observed group was significantly lower than that in the control group (n = 7), P = .04 (Table 4; Fig. 4).

Oswestry Disability Index

The mean ODI of 72.96 ± 8.32% and 71.66 ± 8.09% in the observed group and control group before treatment decreased to 54.70 ± 9.50% and 57.11 ± 9.19% at 3 months post-op (P < .001), 45.44 ± 8.40% and 48.90 ± 8.36% 6 months post-op (P < .001), 34.39 ± 5.93% and 38.46 ± 7.19% 12 months post-op (P < .001), 26.39 ± 5.08% and 31.07 ± 5.34% 36 months post-op (P < .001), and 24.11 ± 5.58% and 32.49 ± 7.65% 72 months post-op (P < .001), respectively. There were no significant differences between the 2 groups at each follow-up except at the last 2 follow-ups (Fig. 5A).

Visual Analog Scale of Pain

The mean VAS of lower limbs declined from a baseline of 7.22 ± 0.73 in the observed group and 7.00 ± 0.81 in the control group to 2.78 ± 1.00 and 2.36 ± 1.00 at 3 months post-op (P < .001), and even further to 1.33 ± 0.49 and 1.00 ± 0.76 at 6 months post-op (P < .001), 0.78 ± 0.73 and 1.27 ± 0.88 at 12 months post-op (P < .001), 0.89 ± 0.68 and 1.68 ± 1.36 at 36 months post-op (P < .001), and 0.89 ± 0.76 and 1.59 ± 0.67 at 72 months post-op (P < .001), respectively. The differences between the 2 groups were statistically significant at 36 months postoperatively (P = .03) and at the last postoperative follow-up (P = .003) (Fig. 5B).

The mean VAS score for LBP in the 2 groups showed nearly the same trend as the mean VAS score of the lower limbs (Fig. 5C).

Japanese Orthopedic Association Score

The mean pretreatment JOA score improved significantly from baseline of 11.33 ± 2.35 and 11.09 ± 1.72 in the observed group and control group before treatment decreased to 14.55 ± 1.63 at 3 months post-op (P < .001), 18.94 ± 1.47 and 18.18 ± 2.22 at 6 months post-op (P < .001), 22.44 ± 1.82 and 21.59 ± 1.47 at 12 months post-op (P < .001), 25.00 ± 1.46 and 23.00 ± 1.63 at 36 months post-op (P < .001), and 26.17 ± 1.79 and 23.91 ± 2.20 at 72 months post-op (P < .001), respectively. Significant differences between the 2 groups were observed at 36 months postoperatively (P < .001) and 72 months postoperatively (P = .001) (Fig. 5D).

The Short Form-36

The SF-36 is composed of 36 items classified into eight categories: physical function, body pain, body role limitations, emotional role limitations, mental health, social...
function, vitality and fatigue, and general health. Out of the items, a physical and a mental health summary score can be calculated (SF-36 physical component score, SF-36 PCS, and SF-36 mental component score, SF-36 MCS).41

The SF-36 PCS improved from the baseline of 21.44 ± 6.46 in the observed group and 21.88 ± 4.83 in the control group to 24.44 ± 6.74 and 24.29 ± 6.09 3 months post-op (P < .001), 40.02 ± 3.32 and 37.74 ± 5.10 6 months post-op (P < .001), 58.28 ± 5.23 and 55.98 ± 4.64 12 months post-op (P < .001), 78.34 ± 5.09 and 74.42 ± 4.79 36 months post-op (P < .001), and 81.09 ± 5.91and 76.51 ± 4.55 72 months post-op (P < .001), respectively. Significant differences between the 2 groups were observed at 36 months postoperatively (P = .017) and 72 months postoperatively (P = .009; Fig. 5E).

The SF-36 MCS also improved significantly from baseline in both groups, and the difference between the 2 groups was also at 36 months postoperatively (P = .001) and 72 months postoperatively (P = .006; Fig. 5F).

Radiological Assessments

**Disc Height Index**

In both groups, the mean DHI of index segments tended toward a reduction, as shown in Fig. 6A. The DHIs in the observed group and control group were 36.68 ± 4.94% and 36.55 ± 4.56% at 3 months post-op, 36.30 ± 4.40% and 36.46 ± 4.35% at 6 months post-op, and 36.22 ± 4.28% and 35.38 ± 4.45% at 12 months post-op, respectively. They were not significantly different from the baseline (36.81 ± 5.19% and 36.89 ± 4.29%). However, the DHI decreased significantly to 34.85 ± 3.94% (P = .099) and 31.97 ± 4.66% (P < .001) at 36 months post-op and to 31.41 ± 4.37%, (P < .001) and 28.38 ± 4.51% (P < .001) at 72 months post-op compared with the baseline, and the decline in the observed group was significantly less than that in the control group.

**Ratio Value of the Grayscale**

Mean RVG in each group showed the same trend as DHI: at baseline, the mean values of RVG in the observed group and control group were 36.81 ± 5.19% and 36.68 ± 4.94%, and 36.55 ± 4.56% at 3 months post-op, 36.30 ± 4.40% and 36.46 ± 4.35% at 6 months post-op, and 36.22 ± 4.28% and 35.38 ± 4.45% at 12 months post-op, respectively. They were not significantly different from the baseline (36.81 ± 5.19% and 36.68 ± 4.94%). However, the DHI decreased significantly to 34.85 ± 3.94% (P = .099) and 31.97 ± 4.66% (P < .001) at 36 months post-op and to 31.41 ± 4.37%, (P < .001) and 28.38 ± 4.51% (P < .001) at 72 months post-op compared with the baseline, and the decline in the observed group was significantly less than that in the control group.

| Table 3. Patient characteristics. |
|----------------------------------|
| **Demographics**                | **Observed group (n = 18)** | **Control group (n = 22)** | **Statistical value** | **P-value)** |
| **Age at surgery (year)**       | 36.39 ± 11.93               | 40.73 ± 12.01               | **t = −1.14**        | **P = .261** |
| **Gender**                      | 16 (88.9%)                  | 16 (72.7%)                  | Fisher’s exact test  | **P = .258** |
| **Operation level**             | 2 (11.1%)                   | 6 (27.3%)                   | **χ² = 2.939**       | **P = .203** |
| **BMI (kg/m²)**                 | 24.68 ± 3.21                | 24.79 ± 2.60                | **t = −0.111**       | **P = .912** |
| **Preoperative Pfirrmann grade**| 5 (27.8%)                   | 9 (40.90%)                  | **t = −1.12**        | **P = .263** |
| **Symptom duration (months)**   | 14.28 ± 6.48                | 16.23 ± 7.01                | **t = −1.14**        | **P = .119** |
| **Cell number ((×10⁶))**        | 5.75 ± 0.60                 | None                        | Fisher’s exact test  | **P = .355** |
| **Follow-up time (median/months)**| 93.23 ± 2.93               | None                        | Fisher’s exact test  | **P = .492** |
| **BMI, body mass index.**       |                             |                             | Fisher’s exact test  | **P = .912** |

| Table 4. Comparison of patients’ complications during the follow-up. |
|-------------------------|------------------------|------------------------|------------------------|------------------------|
| **Complications**       | **Observed Group (n=18)** | **Control Group (n=22)** | **Statistical value** | **P value)** |
| **Low back pain or Lower limbs pain** | 3 (16.67%) | 9 (40.90%) | **χ² = 5.560** | **P = .035** |
| **Disc re-herniation**  | 1 (5.56%)              | 4 (18.18%)             | Fisher’s exact test  | **P = .355** |
| **Lumbar stenosis**     | 0                      | 2 (9.09%)              | Fisher’s exact test  | **P = .492** |
| **Spondylolisthesis**   | 0                      | 1 (4.55%)              | Fisher’s exact test  | **P = .912** |
| **Revision**            | 0                      | 7 (31.82%)             | Fisher’s exact test  | **P = .011** |

Mean RVG in each group showed the same trend as DHI: at baseline, the mean values of RVG in the observed group and control group were 36.68 ± 4.94% and 36.55 ± 4.56% at 3 months post-op, 36.30 ± 4.40% and 36.46 ± 4.35% at 6 months post-op, and 36.22 ± 4.28% and 35.38± 4.45% at12 months post-op, respectively. They were not significantly different from the baseline (36.81 ± 5.19% and 36.68 ± 4.94%). However, the DHI decreased significantly to 34.85 ± 3.94% (P = .099) and 31.97 ± 4.66% (P < .001) at 36 months post-op and to 31.41 ± 4.37%, (P < .001) and 28.38 ± 4.51% (P < .001) at 72 months post-op compared with the baseline, and the decline in the observed group was significantly less than that in the control group.
In the present study, we first conducted a prospective co-
nhort trial to examine the safety of discectomy followed by
autologous discogenic cell transplantation in the treatment of
LDH and the long-term efficacy of discogenic cells for the
treatment of IVDD to demonstrate the prophylactic effect of
discogenic cell transplantation to avoid secondary disease for
up to 6 years after discectomy.

The most important issue that we focused on is the safety
of cell transplantation. In our study, 7 (31.82%) participants
in the control group underwent revision due to accelerated
degeneration of the index disc, which included 4 (18.18%)
disc reherniations, 2 (9.09%) lumbar stenoses, and 1 (4.55%)
spondylolisthesis. The ratio of revision after discectomy in
the long term was in agreement with previous reports.42,43 Outside
of these complications, only a few AEs reported by patients
were determined to be related to the procedure. The majority
of these were due to post-treatment pain that resolved with
conservative care. No neoplasms or heterotopic ossifications
were observed on imaging at the site of cell transplantation,
and no patient developed new neoplastic events after the
procedure. The incidence of lumbar instability in the observed
group was lower than that in the control group.

Compared to the patients treated by discectomy alone,
patients who underwent autologous discogenic cell trans-
plantation followed by discectomy gained the same clinical
improvements in terms of pain and functional scores within
1 year post-treatment, but these scores improved significantly
better at the last 2 visits up to 3 and 6 years after receiving
the treatment. These results demonstrate that discectomy, as
expected, substantially reduced the patients’ disability and
pain. The trend of the sum score continued to improve in the
patients whose treatment was supplemented by cell trans-
plantation, while the control group did not sustain continual
improvement in the long term.

In contrast to patient-reported outcomes, the radiological re-
results (such as DHI and RVG) decreased gradually during the
whole follow-up. Compared with baseline in both groups, the
DHI and RVG of the index discs did not change significantly
during the first year of follow-up, but both parameters decreased
significantly at the last 2 visits (P < .05). Meanwhile, comparison
between groups at the last 2 follow-ups showed that both DHI
and RVG in the observed group decreased less than those in the
control group (P < .05). Autologous discogenic cell transplant-
ation slowed the decreased rate of IVD height and water content.

In addition, although 6 patients in each group underwent
DWI to calculate the ADC value of index levels and adjacent
normal levels at the last follow-up, the mean normalized value
was higher in the observed group (88.83 ± 3.87%) than in the
control group (74.17 ± 10.74%), which is in accordance with the
trend of RVG. These patients also underwent UTE MRI
to assess the morphology of the CEP. Although the number of
CEP defects in the observed group was smaller than that in the
control group, the difference was not significant. All these
radiological results initially demonstrate that discogenic cell
transplantation may have a potential role in retarding further
degeneration after discectomy in the long term.

Discectomy is temporarily effective in eliminating symp-
toms arising from nerve root compression, but it does not
replenish the disc lost from herniation or decelerate the speed
of progression of disc degeneration. Consequently, revision
(such as lumbar fusion) would inevitably be conducted in ad-
ance because of secondary diseases, including reherniation,
spondylolisthesis, spinal stenosis, and ASD.8

Cell therapy could potentially compensate for the shortcom-
ings of discectomy by restoring the normal function of degener-
dated discs. This biological treatment has been demonstrated

### Pfirrmann Grades

According to the Pfirrmann classification system of
T2-weighted sagittal images, there were 3 (16.67%) II, 10
(55.56%) III, 4 (22.22%) IV, and 1 (5.56%) V in the observed
group and 8 (36.36%) III, 10 (45.45%) IV, and 4 (18.18%)
V in the control group at the last follow-up. Pfirrmann grades
in both groups differed significantly from the baseline via
the Wilcoxon signed-rank test. The difference between the
2 groups was also significant at the last follow-up via the
Mann-Whitney U test (P = .019).

### Apparent Diffusion Coefficient Value

At the last follow-up, the mean ADC values of the index levels
were (1.90 ± 0.09) × 10⁻³ mm²/s and (1.63 ± 0.20) × 10⁻³
mm²/s in the observed and control groups, respectively. After
normalization against the ADC value of adjacent normal
levels, the normalized ADC value was 0.89 ± 0.04 in the ob-
served group compared with 0.74 ± 0.11 in the control group
(P = .01), as shown in Fig. 6C.

### Ultrashort Time to Echo MRI

Based on UTE MRI assessment, a total of 24 CEPs (cephalic
and caudal) from 12 IVDs (6 in each group) were evaluated;
4 out of 12 (33.33%) and 7 out of 12 (58.33%) CEPs in the
observed and control groups had defects, but the difference
was not significant (P = .219) (Fig. 7).

### Discussion

In the present study, we first conducted a prospective co-
hort trial to examine the safety of discectomy followed by

![Figure 4. Disc reherniation after the operation in the 2 groups. A1 shows the L5/S1 herniation of one patient in the preoperative observation group. B1 shows the index level 3 months postoperatively. C1 shows reherniation 7 months postoperatively. A2 shows the L4/S5 herniation of one patient in the control group preoperatively. B2 shows the index level 3 months postoperatively. C2 shows the L4/5 herniation 11 months postoperatively. Preop, preoperative; Postop, postoperative; DRH, disc reherniation.](image-url)
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by a large number of animal studies in vivo or in vitro as a promising technique in recent decades and has gradually progressed toward clinical translation. 44,45 Cell therapy is a chronic process, and it cannot relieve clinical symptoms for disc herniated patients with severe neural compression. Therefore, our hypothesis was that if we combine the surgical technique and cell transplantation, we would achieve complete neurological decompression to relieve the symptoms and decelerate disc degeneration to avoid secondary disease after discectomy.

In our study, we collected the patients’ own disc tissue, which is usually abandoned as waste during discectomy, and we cultured the cells to reach a sufficient number for transplantation without concerning autologous IVD tissue unavailability or cell inaccessibility, which are considered hurdles by many scholars.46,47 Meanwhile, this sampling method could avoid another surgical procedure to harvest tissues for transplanted cells, such as bone marrow and adipose tissue, which could lead to donor-site morbidity and patient fear of accepting this technique.48 Nevertheless, not all IVDD is combined with LDH and requires discectomy, so we had the challenge of finding alternatives to autologous discogenic cells for these patients. Additionally, the autologous approach has the benefit of reducing the risk of immunogenicity and avoiding

Figure 5. Clinical efficacy of pain and functional scores over time postoperatively between and within the 2 groups. (A) ODI scores over time. (B and C) The PCS and MCS of SF-36, respectively. (D) The changes of JOA over time. (E and F) The changes in low back pain and lower limb pain assessed by the VAS. JOA, Japanese Orthopedic Association; SF-36, the short form health survey-36; PCS, Physical Health Score; MCS, Mental Health Score; ODI, Oswestry Disability Index; VAS, Visual Analog Scale. a-e, alphabetical labeling of time point differences (comparison within the group), as long as there are the same letters at 2-time points, it means that there is no significant difference between the 2-time points (P > .05), otherwise, different letters at 2-time points mean the difference is significant (P ≤ 0.05). Δ, significant difference in comparison between the 2 groups (comparison between groups).

Figure 6. Results of radiological measurement. (A) Changes in DHI during the follow-up. (B) Changes in RVG during the follow-up. (C) The difference in ADC value at last follow-up between the 2 groups. DHI, disc height index. RVG, ratio value of the grayscale. ADC, the apparent diffusion coefficient. a-e, Alphabetical labeling of time point differences (comparison within the group). Δ, significant difference in comparison between the 2 groups (comparison between groups).
Anchored mesenchymal stem cells (BM-MSCs) in re-
NP-derived stem cells possess the same characteristics as bone
from degenerated discs. Our previous study also isolated
immediately after the operation, without a significant differ-
ethical problems, although the environment within the IVD is
immuno-deprived.
In our study, both groups gained significant improvement immediately after the operation, without a significant difference between the 2 groups within the first year. These results were undoubtedly due to the complete neural decompression of the decocation, but the maintenance of the efficacy and the difference in revision at 36 months or even 72 months of follow-up could be attributed to discogenic cell transplanta-
tion. The better results of radiological assessment in the ob-
served group could further demonstrate that discogenic cells may have the potential for degenerative disc repair.
One fundamental mechanism would be that progenitor cells or stem cells in the disc tissue is activated via GMP culture and maintains their regenerative ability. Since 2007, Rishud et al first reported that degenerated human discs contain skeletal progenitors. In 2010, Blanco first isolated stem cells from degenerative discs. Our previous study also isolated NP-derived stem cells from degenerated NP and revealed that NP-derived stem cells possess the same characteristics as bone marrow derived mesenchymal stem cells (BM-MSCs) in regen-
eration ability. Our other study also demonstrated the existence of NP-derived mesenchymal stem cell (NP-MSCs) in both degenerative and nondegenerative NP tissue, thus providing evidence for the presence of stem cells in degener-
ated NP and revealing the potential regeneration ability of discogenic cells. Other previous studies also described the presence of stem cells and progenitor cells in the tissue of the IVD.
Another possible mechanism may be related to the inter-
action among the 3 kinds of discogenic cells after transplanta-
tion. In our study, AF and CEP tissues were not deliberately removed in the process of tissue separation to retain the 3 kinds of cells. In 2000, Okuma et al conducted coculture of NP cells and AF cells using the same medium and demonstrated that coculture of the 2 cell types stimulated prolif-
eration of each. In 2016, Wang et al also used the same method of acquisition and culture of NP cells, AF cells, and CEP cells to study the differences in the biological charac-
teristics of these and bone marrow-derived mesenchymal stem cells. Many other related studies also applied the same method of harvesting and culturing these 3 kinds of cells or NP cells alone to examine their biological characteristics and differ-
ences. In fact, most studies cannot separate NP tissue from AF tissue or CEP tissue completely in the process of tissue isolation under a dissecting microscope or even the naked eye, especially in degenerated discs that have vague boundaries between the NP and AF. In addition, no study thus far has identified NP cells, AF cells, and CEP cells through simple morphological observation. Based on this, we believe that the transplanted cells contain 3 kinds of cells. In our future study, we will further focus on the cellular types and characteristics.
The maintenance of biomechanical function and integrity of the disc is independent of the metabolic balance of NP cells, AF cells, and CEP cells and their paracrine effects to stimulate endogenous cells or transplanted cells to produce a neomatrix. Theoretically, the reinsertion of endogenous cells into the disc is more advantageous than exogenous cells to adapt to the hostile milieu of the disc. Moreover, many studies have demonstrated that not only does the NP tissue contain stem cells but also AF and EP tissue. Overall, transplanting discogenic cells seem to have an absolute advantage over other single-type cell transplantations in the treatment of IVDD.
We have also hypothesized the following mechanism. Based on the existence of stem cells in the disc, the cultured discogenic cells are reintersorted into the index disc. These sufficient cells may contain a certain amount of stem cells and play their own repair role, including NP cells synthesizing extracellular matrix, AF cells repairing defects, CEP cells repairing injuries, and finally maintaining the structural and functional integrity of the intervertebral disc through the interaction of the 3 cells. On the other hand, disc degeneration is a natural biological process related to age, and even when performing cell transplantation to such a disc with prevention in mind, the progression of disc degeneration inevitably follows its natural course. However, transplanting autologous discogenic cells has the potential to retard the traumatic acceleration of disc degeneration after discomy.
In this study, we not only used traditional measurement methods, such as intervertebral disc height and intervertebral disc grayscale but also used quantitative methods, such as the ADC value, to evaluate IVDD. Because of the small sample size and individual differences among patients, DHI, RVG, and normalized ADC values were selected to compare the degree of IVDD between the 2 groups. Pfirrmann classification, as a qualitative analysis, is still the mainstream method to evaluate IVDD in clinical practice worldwide. However, this classification system is very subjective and ambiguous due to the lack of a quantitative index, and it cannot be used to assess emerging cell-based therapies for IVDD. Magnetic resonance imaging (MRI), an accurate and noninvasive imaging technique, has made great progress in clinical diag-
nosis. Quantitative imaging has also received more attention mainly because of the relative objectivity in structure change detection, including T1rho imaging, T2 mapping, T2* mapping, diffusion-weighted imaging (DWI), diffusion tensor imaging, and ultrashort time to echo DWI, diffusion-weighted images ADC, apparent diffusion coefficient. We have also hypothesized the following mechanism.
imaging, and chemical exchange saturation transfer.\textsuperscript{19-63} These recently developed quantitative MRI techniques are useful for detecting some imperceptible biochemical changes within the discs.\textsuperscript{64} However, the sensitivity and accuracy of these methods remain controversial. Kumar’s and Mochida’s studies,\textsuperscript{29,66} both of which were clinical trials to assess the efficacy of cell-based therapies for degenerative disc disease, used ADC mapping from DWI to determine the water content of treated discs and confirmed that the ADC value was more reliable to assess subtle changes in water content. Therefore, at the last follow-up, our study also attempted to use the ADC value to compare disc hydration between the 2 groups, and the results were consistent with traditional methods. Our future research will be conducted to explore the potential advantages of DWI in the assessment of disc degeneration.

Our study was the first to apply the UTE technique to evaluate the morphology of CEPs to indirectly assess the long-term efficacy of cell therapy in the treatment of IVDD. As described previously, the intervertebral disc is a 3-structure complex, cartilaginous endplates play an important role in the function and homeostasis of the disc by serving as a mechanical stabilizer as well as a pathway for nutrient transport, and its structural and compositional changes are closely related to disc degeneration.\textsuperscript{65} UTE MRI, a novel imaging approach, can assess MRI signals from short-T2 cartilaginous endplates that are not detected on conventional T2W MRI, thus complementing the limitations of conventional T2W MRI in assessing the integrity of lumbar discs and their clinical relevance.\textsuperscript{66,67} Many in vitro and in vivo studies have demonstrated a significant association between the presence of CEP defects and disc degeneration.\textsuperscript{40,68,69} Therefore, the evaluation of CEP abnormalities by UTE MRI can provide a subtle reference for IVD degeneration. UTE MRI is also regarded as an important tool to select appropriate patients for biological therapies of disc repair in addition to predicting individuals at high risk for IVD.\textsuperscript{70} Regrettably, our study lacks preoperative UTE imaging data in each group, and there has not been a definite index or grading system of UTE imaging to assess the degree of disc degeneration. However, our report aims to raise awareness of UTE as a unique imaging biomarker to provide complementary information to that provided by conventional T1W or T2W MRI techniques, which we hope will contribute to the accuracy of disease diagnosis and the science of treatment.

At present, we should give more attention to defining the precise and optimal cell number required for functional regeneration, which is also an issue specified in a variety of studies.\textsuperscript{71-72} The harsh microenvironment within the disc is a potential limitation of cell-based regeneration and deteriorates in the process of degeneration. Transplanting a large concentration of cells could compete for already limited nutrients, potentially inducing more cell death and senescence and further skewing the imbalance toward degeneration.\textsuperscript{73} In contrast, insufficient cells cannot withstand the hostile milieu to keep viable and function ideally. Thus, our study chose a treatment amount of $10^6$ based on the experience of the above studies.

Another crucial issue we should pay attention to is cell leakage, which may result in adverse effects, such as osteophyte formation.\textsuperscript{74,75} Annular puncture plus discectomy defects have been demonstrated to induce degeneration if the transplanted cells fail to function well.\textsuperscript{76,77} However, in our study, intradiscal puncture was traditionally adopted to deliver cells without carriers or scaffolds, and no patients showed AEs related to cell leakage upon imaging examination. The primary cause of cell leakage may be the high IVD pressure with contact AF. The initial discectomy in the observed group created an “artificial cavity”, although it introduced a defect of AF at the same time, which greatly reduced the internal pressure when transplanting discogenic cells. In addition, we applied a 22-gauge puncture needle through the opposite side of the discectomy and stayed for a few minutes to maintain more cell retention, all of which can decrease the risk of cell leakage. Another mechanism is that the AF cells in the transplanted cells could home to the defective site to repair AF-like endogenous cells as well as mesenchymal stem cell homing for disc regeneration according to the studies of Wangler and Grad.\textsuperscript{78-79}

However, with a relatively small sample size, which may lead to a certain deviation, caution must be applied, as the generalization of our results might be limited. Given that the lack of a control group and long-term follow-up are the main limitations in most of the clinical studies to date, we conducted this controlled, long-term clinical trial to further verify the real efficacy of intradiscal cell transplantation and achieved favorable outcomes.

**Conclusion**

Discectomy combined with autologous discogenic cell transplantation to treat symptomatic LDH produced encouraging results in this controlled study. The results also showed a lower incidence of AEs and revision, substantially reduced pain, and increased function. Radiological analysis showed an initial trend of beneficial outcomes favoring cell transplantation. Surgery in conjunction with biological therapy can be regarded as a novel treatment concept to achieve symptom relief and prevention of secondary disease simultaneously.

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**Conflict of Interest**

The authors declared no conflicts of interest.

**Author Contributions**

D.R., A.X., C.W.: conception/design, data analysis and interpretation, manuscript writing. Q.H., D.R.: surgical management, provision of study material or patients. A.X., Z.Z., D.W., C.X.: data collection and analysis, drafting the
manuscript. L.H., C.Z., C.L., W.J., T.W.: data measurement, data analysis and interpretation, manuscript writing. C.W., W.J., T.W.: cell culture and counting, preparation of cell suspension. All authors have read, revised, and approved the submitted manuscript.

Ethics Declarations
Ethics approval and consent to participate. Ethics committee approval for cell transplantation after discectomy was obtained from the Ethics Committee of the Sixth Medical Center of PLA General Hospital (Beijing, China) (HZKY-PJ-2021-18). The study was performed according to the tenets of the 2013 Declaration of Helsinki. Written informed consent was obtained from all the patients prior to enrollment.

Data Availability
The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

References
1. Foster NE, Anema JR, Cherkin D, et al. Lancet Low Back Pain Series Working Group. Prevention and treatment of low back pain: evidence, challenges, and promising directions. Lancet. 2018;391(10137):2368-2383.
2. Cieza A, Causey K, Kamenov K, Hanson SW, Chatterji S, Vos T. Global estimates of the need for rehabilitation based on the Global Burden of Disease study 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2021;396(10267):2006-2017.
3. Alabbas FM, Almatrafi FR, Elshawarby M. The correlation between histopathology of herniated lumbar intervertebral disc and clinical findings. Asian J Neurosurg. 2020;15(3):545-553.
4. Pattappa G, Li Z, Peroglio M, Wismer N, Alini M, Grad S. Diversity of intervertebral disc cells: phenotype and function. J Anat. 2012;221(6):480-496.
5. Lyu FJ, Cheung KM, Zheng Z, et al. IVD progenitor cells: a new horizon for understanding disc homeostasis and repair. Nat Rev Rheumatol. 2019;15:102-112.
6. Chen BL, Guo JB, Zhang HW, et al. Surgical versus non-operative treatment for lumbar disc herniation: a systematic review and meta-analysis. Clin Rehabil. 2018;32(2):146-160.
7. Saal JA, Saal JS. Nonoperative treatment of herniated lumbar intervertebral disc with radiculopathy. An outcome study. Spine (Phila Pa 1976). 1989;14(11):130-137.
8. Alvi MA, Kerezoudis P, Wahood W, Goyal A, Bydon M. Operative approaches for lumbar disc herniation: a systematic review and multiple treatment meta-analysis of conventional and minimally invasive surgeries. World Neurosurg. 2018;114:391-407.e2.
9. Ajiboye RM, Drysch A, Mosich GM, Sharma A, Pourhakimer S. Surgical treatment of recurrent lumbar disk herniation: a systematic review and meta-analysis. Orthopedics. 2018;41(4):e457-e469.
10. Nguyen TH, Randolph DC, Talmage J, Succop P, Travis R. Long-term outcomes of lumbar fusion among workers’ compensation subjects: a historical cohort study. Spine (Phila Pa 1976). 2011;36(4):320-331.
11. Min JH, Jang JS, Lee SH. Comparison of anterior- and posterior-approach instrumented lumbar interbody fusion for spondyloolisthesis. J Neurosurg Spine. 2007;7(1):21-26.
12. Abi-Hanna D, Kerferd J, Phan K, Rao P, Mobbs R. Lumbar disk arthroplasty for degenerative disk disease: literature review. World Neurosurg. 2018;109:188-196.
13. Ding F, Jia Z, Zhao Z, et al. Total disc replacement versus fusion for lumbar degenerative disc disease: a systematic review of overlapping meta-analyses. Eur Spine J. 2017;26(3):806-815.
14. Thalhammer T, Altmeyer TM, Woratschek H, et al. Minimally invasive discectomy for lumbar disc herniation: current concepts, surgical techniques, and outcomes. Int Orthop. 2019;43(4):917-922.
15. Wu T, Song HX, Dong Y, Li JH. Cell-based therapies for lumbar discogenic low back pain: systematic review and single-arm meta-analysis. Spine (Phila Pa 1976). 2018;43(1):49-57.
16. Sun Y, Leung YV, Cheung KM. Clinical trials of intervertebral disc regeneration: current status and future developments. Int Orthop. 2019;43(4):1003-1010.
17. Wang W, Deng G, Qiu Y, et al. Transplantation of allogenic nucleus pulposus cells attenuates intervertebral disc degeneration by inhibiting apoptosis and increasing migration. Int J Mol Med. 2018;41(5):2553-2564.
18. Clouet J, Fuselier M, Camus A, Le Visage C, Guicheux J. Intervertebral disc regeneration: From cell therapy to the development of novel bioinspired endogenous repair strategies. Adv Drug Deliv Rev. 2019;146:306-324.
19. Elabd C, Centeno CJ, Schultz JR, et al. Intradiscal injection of autologous, hypoxic cultured bone marrow-derived mesenchymal stem cells in five patients with chronic lower back pain: a long-term safety and feasibility study. J Transl Med. 2016;14:253.
20. Mochida J, Sakai D, Nakamura T, Watanabe T, Yamamoto Y, Kato S. Intervertebral disc repair with activated nucleus pulposus cell transplantation: a three-year, prospective clinical study of its safety. Eur Cell Mater. 2015;29:202-212; discussion 212.
21. Kumar H, Ha DH, Lee EJ, et al. Safety and tolerability of intradiscal implantation of combined autologous adipose-derived mesenchymal stem cells and hyaluronic acid in patients with chronic discogenic low back pain: 1-year follow-up of a phase I study. Stem Cell Res Ther. 2017;8:262.
22. Comella K, Silbert R, Parlo M. Effects of the intradiscal implantation of stromal vascular fraction plus platelet rich plasma in patients with degenerative disc disease. J Transl Med. 2017;15(1):12.
23. Nishimura K, Mochida J. Percutaneous reinsertion of the nucleus pulposus. An experimental study. Spine (Phila Pa 1976). 1998;23(14):1531-8; discussion 1539.
24. Hohus C, Ganey TM, Meisel Y, et al. Cell transplantation in lumbar spine disc degeneration disease. Eur Spine. 2008;492-503.
25. Meisel HJ, Siodla V, Ganey T, Minkus Y, Hurton WC, Alasevic OJ. Clinical experience in cell-based therapeutics: disc chondrocyte transplantation A treatment for degenerated or damaged intervertebral disc. Biomol Eng. 2007;24(1):5-21.
26. Meisel HJ, Ganey T, Hurton WC, Libera J, Minkus Y, Alasevic O. Clinical experience in cell-based therapeutics: intervention and outcome. Eur Spine J. 2006;15 Suppl 3:S397-S405.
27. Tschugg A, Diepers M, Simone S, et al. A prospective randomized multicenter phase I/II clinical trial to evaluate safety and efficacy of NOVOCART disk plus autologous disk chondrocyte transplantation in the treatment of nucleotomized and degenerative lumbar disks to avoid secondary disease: safety results of Phase I-a short report. Neurosurg Rev. 2017;40(1):155-162.
28. Tschugg A, Michnaes F, Strowitzki M, Meisel HJ, Thomé C. A prospective multicenter phase III clinical trial to evaluate safety and efficacy of NOVOCART Disc plus autologous disc chondrocyte transplantation in the treatment of nucleotomized and degenerative lumbar disk to avoid secondary disease: study protocol for a randomized controlled trial. Trials. 2016;17(1):108.
29. Jia Z, Yang P, Wu Y, et al. Comparison of biological characteristics of nucleus pulposus meniscal stem cells derived from non-degenerative and degenerative human nucleus pulposus. Exp Ther Med. 2017;13(6):3574-3580.
31. Wang H, Zhou Y, Chu TW, et al. Distinguishing characteristics of stem cells derived from different anatomical regions of human degenerated intervertebral discs. *Eur Spine J.* 2016;25:2691-2704.

32. Liu IT, Huang B, Li CQ, Zhuang Y, Wang J, Zhou Y. Characteristics of stem cells derived from the degenerated human intervertebral disc cartilage endplate. *PLoS One.* 2011;6(10):e26285.

33. Feng G, Yang X, Shang H, et al. Multipotential differentiation of human anulus fibrosus cells: an in vitro study. *J Bone Joint Surg Am.* 2010;92(3):675-685.

34. Blanco JF, Graciani IF, Sanchez-Guijo FM, et al. Isolation and characterization of mesenchymal stromal cells from human degenerated nucleus pulposus: comparison with bone marrow mesenchymal stromal cells from the same subjects. *Spine (Phila Pa 1976).* 2010;35(26):2259-2265.

35. Fairbank JC, Pyne PB. The Oswestry disability index. *Spine (Phila Pa 1976).* 2000;25:2940-2952; discussion 2952; discussion 52.

36. Patel AA, Donegan D, Albert T. The 36-item short form. *J Am Acad Orthop Surg.* 2007;15(2):126-134.

37. Azimi P, Mohammadi HR, Montazeri A. An outcome measure of functionality and pain in patients with lumbar disc herniation: a validation study of the Japanese Orthopedic Association (JOA) score. *J Orthop Sci.* 2012;17(4):341-345.

38. Ying J, Han Z, Zeng Y, et al. Evaluation of intervertebral disc regeneration with injection of mesenchymal stem cells encapsulated in PEGDA-microcryogel delivery system using quantitative T2 mapping: a study in canines. *Am J Transl Res.* 2019;11(4):2028-2041.

39. Pfirrmann CW, Metzdorf A, Zanetti M, Hodler J, Boos N. Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine (Phila Pa 1976).* 2001;26(17):1873-1878.

40. Law T, Anthony MP, Chan Q, et al. Ultrashort time-to-echo MRI of the cartilaginous endplate: technique and association with intervertebral disc degeneration. *J Med Imaging Radiat Oncol.* 2013;57(4):427-434.

41. Ware JE Jr, Gandek B. Overview of the SF-36 health survey and the international quality of life assessment (IQOLA) project. *J Clin Epidemiol.* 1998;51(11):903-912.

42. Cheng J, Wang H, Zheng W, et al. Reoperation after lumbar disc surgery in two hundred and seven patients. *Int Orthop.* 2013;37(8):1511-1517.

43. Heindel P, Tuchman A, Hsieh PC, et al. Reoperation rates after single-level lumbar discectomy. *Spine (Phila Pa 1976).* 2014;42(8):E496–E501.

44. Sakai D, Andersson GB. Stem cell therapy for intervertebral disc regeneration: obstacles and solutions. *Nat Rev Rheumatol.* 2015;11:243-256.

45. Benneker LM, Andersson G, Iatridis JC, et al. Cell therapy for intervertebral disc repair: advancing cell therapy from bench to clinics. *Eur Cell Mater.* 2014;27:5-11.

46. Dowdell J, Erwin M, Choma T, et al. Intervertebral disc degeneration and repair. *Neurosurgery.* 2017;80:S46–S54.

47. Sakai D, Nakamura Y, Nakai T, et al. Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. *Nat Commun.* 2012;3:1264.

48. Piccirilli M, Delfinis CP, Santoro A, Salvati M. Mesenchymal stem cells in lumbar spine surgery: a single institution experience about red bone marrow and fat tissue derived MSCs. *J Neurosurg Sci.* 2017;61(2):124-133.

49. Raszud MV, Guttapalli A, Tsai TT, et al. Evidence for skeletal progenitor cells in the degenerate human intervertebral disc. *Spine (Phila Pa 1976).* 2007;32(23):2537-2544.

50. Hauksson H, Thornemo M, Karlsson G, et al. Identification of cell proliferation zones, progenitor cells and a potential stem cell niche in the intervertebral disc region: a study in four species. *Spine (Phila Pa 1976).* 2009;34(21):2278-2287.

51. Okuma M, Mochida J, Nishimura K, Sakabe K, Seiki K. Reinnervation of stimulated nucleus pulposus cells retards intervertebral disc degeneration: an in vitro and in vivo experimental study. *J Orthop Res.* 2000;18(6):988-997.

52. De Luca P, Castagnetta M, de Girolamo L, et al. Intervertebral disc and endplate cell characterisation highlights annulus fibrosus cells as the most promising for tissue-specific disc degeneration therapy. *Eur Cells Mater.* 2020;39:156-170.

53. Liu S, Liang H, Lee SM, Li Z, Zhang J, Fei Q. Isolation and identification of stem cells from degenerated human intervertebral discs and their migration characteristics. *Acta Biochim Biophys Sin (Shanghai).* 2017;49(2):101-109.

54. Liang L, Li X, Li D, et al. The characteristics of stem cells in human degenerative intervertebral disc. *Medicine (Baltimore).* 2017;96(25):e7178.

55. Sato M, Asazuma T, Ishihara M, et al. An experimental study of the regeneration of the intervertebral disc with an allograft of cultured annulus fibrosus cells using a tissue-engineering method. *Spine.* 2003;28:548-553.

56. Wang H, Zhou Y, Chu TW, et al. Distinguishing characteristics of stem cells derived from different anatomical regions of human degenerated intervertebral discs. *Eur Spine J.* 2016;25(9):2691-2704.

57. Iatridis JC, Nicoll SB, Michalek AJ, et al. Biomechanics in annulus fibrosus degeneration and regeneration. *Adv Exp Med Biol.* 2018;1078:409-420.

58. Urrutia J, Besa P, Campos M, et al. The Pfirrmann classification of lumbar intervertebral disc degeneration: an independent inter-and intra-observer agreement assessment. *Eur Spine J.* 2016;25(9):2728-2733.

59. Han Z, Gao L, Shi Q, et al. Quantitative magnetic resonance imaging for diagnosis of intervertebral disc degeneration of the cervico-thoracic junction: a pilot study. *Am J Transl Res.* 2018;10(3):925-935.

60. Yoon MA, Hong SJ, Kang CH, Ahn KS, Kim BH. T1rho and T2 mapping of lumbar intervertebral disc: correlation with degeneration and morphologic changes in different disc regions. *Magn Reson Imaging.* 2016;34(7):932-939.

61. Shen S, Wang H, Zhang J, Wang F, Liu SR. Diffusion weighted imaging, diffusion tensor imaging, and T2* mapping of lumbar intervertebral disc in young healthy adults. *Iran J Radiol.* 2016;13(1):e30069.

62. Wu N, Liu H, Chen J, et al. Comparison of apparent diffusion coefficient and T2 relaxation time variation patterns in assessment of age and disc level related intervertebral disc changes. *PLoS One.* 2013;8(7):e69052.

63. Kerttula L, Kurunlahti M, Jauhiainen J, Koivula A, Oikarinen J, Tervonen O. Apparent diffusion coefficients and T2 relaxation time measurements to evaluate disc degeneration. A quantitative MR study of young patients with previous vertebral fracture. *Acta Radiol.* 2018;10(3):925-935.

64. Cao Y, Guo QW, Wan YD. Significant association between the Pfirrmann classification and endplate cell characterisation highlights annulus fibrosus cells as the most promising for tissue-specific disc degeneration therapy. *Eur Cells Mater.* 2020;39:156-170.

65. Moore RJ. The vertebral endplate: what do we know? *Eur Spine J.* 2020;9(2):92-96.

66. Bae WC, Statum S, Zhang Z, et al. Morphology of the cartilaginous endplates in human intervertebral disks with ultrashort echo time MR imaging. *Eur Spine J.* 2016;25(9):2691-2704.

67. Moore RJ. The vertebral endplate: what do we know? *Eur Spine J.* 2020;10(2):444-454.

68. Ing X, Zhou Z, Figini M, Shangguan J, Zhang Z, Chen W. Multi-parameter evaluation of lumbar intervertebral disc degeneration using quantitative magnetic resonance imaging techniques. *Am J Transl Res.* 2018;10(2):444-454.

69. Ing X, Zhou Z, Figini M, Shangguan J, Zhang Z, Chen W. Multi-parameter evaluation of lumbar intervertebral disc degeneration using quantitative magnetic resonance imaging techniques. *Am J Transl Res.* 2018;10(2):444-454.

70. Ing X, Zhou Z, Figini M, Shangguan J, Zhang Z, Chen W. Multi-parameter evaluation of lumbar intervertebral disc degeneration using quantitative magnetic resonance imaging techniques. *Am J Transl Res.* 2018;10(2):444-454.
70. Chang EY, Du J, Chung CB. UTE imaging in the musculoskeletal system. *J Magn Reson Imaging*. 2015;41(4):870-883.

71. Takafumi Yoshikawa M, Yurito Ueda M, Kiyoshi Miyazaki M, et al. Disc regeneration therapy using marrow mesenchymal cell transplantation. *Spine*. 2010;35:E475-E480.

72. Haufe SMW, Anthony RM. Intradiscal injection of hematopoietic stem cells in an attempt to rejuvenate the intervertebral discs. *Stem Cells Dev*. 2006;15:136-137.

73. Smith LJ, Silverman L, Sakai D, et al. Advancing cell therapies for intervertebral disc regeneration from the lab to the clinic: Recommendations of the ORS spine section. *J OR Spine*. 2018;1:e1036.

74. Vadalà G, Sowa G, Hubert M, Gilbertson LG, Denaro V, Kang JD. Mesenchymal stem cells injection in degenerated intervertebral disc: cell leakage may induce osteophyte formation. *J Tissue Eng Regen Med*. 2012;6(5):348-355.

75. Schol J, Sakai D. Cell therapy for intervertebral disc herniation and degenerative disc disease: clinical trials. *Int Orthop*. 2019;43(4):1011-1025.

76. Michalek AJ, Buckley MR, Bonassar LJ, Cohen I, Iatridis JC. The effects of needle puncture injury on microscale shear strain in the intervertebral disc annulus fibrosus. *Spine J*. 2010;10(12):1098-1105.

77. Vergari C, Mansfield JC, Chan D, Clarke A, Meakin JR, Winlove PC. The effects of needle damage on annulus fibrosus micromechanics. *Acta Biomater*. 2017;63:274-282.

78. Wangler S, Peroglio M, Menzel U, et al. Mesenchymal stem cell homing into intervertebral discs enhances the Tie2-positive progenitor cell population, prevents cell death, and induces a proliferative response. *Spine (Phila Pa 1976)*. 2019;44(23):1613-1622.

79. Grad S, Peroglio M, Li Z, Alini M. Endogenous cell homing for intervertebral disk regeneration. *J Am Acad Orthop Surg*. 2015;23(4):264-266.