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Autologous adipose-derived mesenchymal stem cells combined with hyaluronic acid hydrogel in the treatment of discogenic low back pain: a study protocol for a phase II randomized controlled trial

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ABSTRACT

Introduction: Discogenic low back pain (DLBP) is a common disease, and its occurrence is closely related to intervertebral disc degeneration (IDD). At present, none of the traditional treatment methods can repair the degenerated intervertebral disc (IVD). The emergence of stem cell therapy makes it possible to repair and regenerate IVDs tissue, among which adipose-derived mesenchymal stem cells (AD-MSCs) transplantation therapy has become a hot spot of current research. Therefore, this trial aimed to investigate the safety and efficacy of using autologous AD-MSCs combined with hyaluronic acid (HA) hydrogel in the treatment of DLBP.

Methods and analysis: This study is a randomized, dose-escalation, placebo-controlled, double-blind, single-center, Phase II clinical trial to evaluate the efficacy and safety of autologous AD-MSCs combined with HA hydrogel in the treatment of patients with DLBP. The 100 eligible patients will be randomly divided into 3 experimental groups with different doses and 1 placebo control group in a ratio of 1:1:1:1. All patients will undergo liposuction to obtain ADMSCs, followed by autologous AD-MSCs mixtures or placebo transplantation after three weeks. The patients will be followed up to 24 months after the transplant. The primary endpoint of this trial is the visual analogue scale (VAS). Secondary endpoints include Oswestry disability index (ODI), Japanese orthopaedic association (JOA) scores, the Mos 36-item short form (SF-36), the Modic classification, Pfirrmann grade, height and segment range of motion of the IVD, vital signs (temperature, pulse, respiration, blood pressure), blood routine, liver and kidney function, immunological examination, urinalysis, and treatment emergent adverse events.

Ethics and dissemination: The study protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University and registered in Chinese Clinical Trial Registry. Dissemination of the results will be presented at a conference and in peer-reviewed publications.

Trial registration: ChiCTR2200058291

KEYWORDS

Protocols & guidelines; Back pain; Spine

Strengths and limitations of this study

► This study is a randomized, placebo-controlled, double-blind trial, so bias will be minimized.

► The results of this study are expected to determine the optimal therapeutic dose for transplantation of AD-MSCs into the degenerative IVD by dose-escalation.

► The subjects of this trial are recruited from only one research center, and the sample size is not large enough.

► The subjects of this trial are patients with DLBP with single disc segment degeneration, which would limit applicability to the general population.

INTRODUCTION

Low back pain (LBP) is a common health concern worldwide. According to some studies, the point prevalence of LBP is 11.9%, and the one-month prevalence is 23.3%, which is the main reason for years lived with disability counts and puts a heavy economic burden on patients and society[1-3]. Discogenic low back pain (DLBP) is a common source of LBP, with an overall prevalence of 26%~42%, and in the younger population, this rate reaches more than 80%[4,5]. DLBP gradually evolves from internal intervertebral disc (IVD) diseases such as inflammation, deformation and annulus fibrosus (AF) injury, and its key pathological process is IVD degeneration (IDD)[6,7]. The clinical manifestations of patients with DLBP are recurrent LBP, especially when sitting for a long time, bending over or coughing, but there is often no positive feature of nerve root damage during physical examination. Although the pathological mechanism of DLBP has not been fully understood, it is widely believed that the sensory nerve fibers from the outer layer of the IVD grow into the interior through the fissure, and the production of proinflammatory cytokines in the degenerated IVD

increases, resulting in back pain in patients[8-10]. In addition, abnormal lumbar disc activity due to long-term mechanical injury may accelerate the progression of DLBP[11,12]. The diagnosis of DLBP requires a combination of imaging findings and long-term recurrent LBP. Clinically, magnetic resonance imaging (MRI) is an essential tool for evaluating intervertebral disc pathology, and its signal characteristics reflect the degree of IVD aging or degeneration[7]. Many patients with DLBP are found to have decreased signal intensity in the IVD on MRI T2-weighted imaging, which is the so-called "black" IVD, and the sagittal view shows that the posterior border of the IVD has a small, round, limited area of high signal intensity[13,14]. However, the relationship between the degree of disc degeneration on imaging and the severity of pain remains unclear, so it is necessary to rely on CT-guided discography for further diagnosis. Discography is currently the only "gold standard" test for diagnosing DLBP. Following injection of the contrast agent into the disc, patients will experience similar pain responses, but the adjacent disc will not[14]. Based on discography, DLBP may be categorized as AF ruptured LBP and CEPs ruptured LBP, and this classification method has clinical and theoretical support[15]. Due to the varying severity of clinical manifestations in patients with DBLP, step-by-step therapy is often used in the treatment. Traditional treatment methods include conservative, interventional, and surgical treatments[15]. Most patients tend to opt for conservative treatments due to mild symptoms and a short course of the disease, including bed rest, physiotherapy with microwave or infrared, oral painkillers, and functional exercises. Interventional treatments, such as epidural injections and percutaneous intradiscal therapies, are generally performed if conservative treatments fail[16-18]. With severe symptoms or ineffective conservative and interventional treatments, surgical treatments are often recommended. The most commonly used surgical procedures are interbody fusion and artificial disc replacement[18]. However, conservative treatments have limited efficacy, and interventional treatments for pain relief should be further evaluated. Although surgical treatments can effectively relieve pain, they may cause complications such as infection, nerve damage, large blood vessel damage and adjacent segment degeneration due to improper operation or care, which will further damage the body of patients[19,20].

Additionally, the best way to treat DLBP would be to slow down or even reverse the process of IDD, but neither conservative treatments, interventional treatments nor surgical treatments can do anything about it. Traditional treatments are in a dilemma, and a new treatment is urgently needed to induce repair of degenerated disc tissue.

The IVD is composed of an outer AF, a nucleus pulposus (NP) in the middle, and cartilage endplates (CEPs) at the upper and lower ends. NP is the main structure of IVD, which is mainly composed of NP cells and extracellular matrix (ECM). NP cells are cartilage-like cells. The main components of ECM are COL II, proteoglycans and other matrix proteins[21]. IDD mainly occurs in NP. With the degradation of ECM and the loss of proteoglycans, these changes will reduce the structural integrity of IVD and eventually damage its function[22]. The recent deepening of the understanding of IDD has promoted the research of biological therapy, among which stem cell therapy stands out and becomes the current research hotspot. In the past few decades, human stem cell therapy indications have involved many different fields, including neurological diseases, cardiovascular diseases, diabetes, blood diseases and cancer, with exciting results[23-26]. Given the broad application prospects of stem cell therapy in regenerative medicine, people have begun to explore the application in spinal degenerative diseases. DLBP is a common spinal degenerative disease, and the key to its treatment is to repair the degenerated disc tissue and reduce the discomfort of patients. The transplanted stem cells are capable of self-replication, renewal, and multi-directional differentiation, which can differentiate into chondrocytes to replace the lost NP cells, as well as promoting the formation of ECM by co-culturing with NP cells[27-29]. In addition, stem cells have immunomodulatory effects and can secrete various cytokines to improve the microenvironment inside the IVD, promote the repair of degenerated IVD tissue, and relieve the pain of patients[27-29]. Stem cells for the treatment of IDD come from a wide range of sources, including bone marrow mesenchymal stem cells (BM-MSCs) and adipose-derived MSCs (AD-MSCs), and a few stem cell types derived from human umbilical cord MSCs (HUC-MSCs), pluripotent stem cells (PSCs), NP-derived stem cells (NPSCs) and other sources[29,30]. Among the many types of stem cells, BM-MSCs are currently the most studied, and their safety and efficacy in the treatment of IDD

have been verified in clinical trials, bringing the dawn of stem cell clinical treatment for IDD[31,32]. However, due to the cumbersome and invasive process of obtaining BM-MSCs, its clinical application is limited to a certain extent[33,34]. With the deepening of research, it has been found that AD-MSCs have similar chondrogenic differentiation potential compared with BM-MSCs. More importantly, AD-MSCs have the advantages of easy to obtain in large quantities, lower incidence of donor site and higher proliferation potential, so they may be an ideal source of stem cells for the treatment of DLBP[33,35].

During stem cell therapy, potential complications such as leakage and osteophyte formation may occur at the infusion site, and the implanted stem cells are subject to high mechanical loads in the disc, which may reduce the viability or function of the stem cells, resulting in affect the treatment effect. In order to solve these problems, various scaffolds have been designed as carriers for delivering stem cells, among which hydrogel scaffolds are more commonly used[36-38]. Among various biomaterials for the production of hydrogel scaffolds, HA and its derivatives have been extensively studied. HA is a naturally occurring glycosaminoglycan that is involved in vital processes such as cell proliferation, migration, angiogenesis, and tissue growth. Biocompatibility, biodegradability, processability, and tunable mechanical properties of HA contribute to its clinical appeal[39-41]. Therefore, the transplantation of AD-MSCs combined with HA hydrogel has become a hot research topic.

The efficacy of AD-MSCs in the treatment of DLBP has been verified in animal models[42-44]. In order to further verify whether stem cell therapy is also safe and effective in humans, it is necessary to conduct clinical trials. Currently, five clinical trials of AD-MSCs in the treatment of IDD are registered on the ClinicalTrials.gov website, and three of them (NCT01643681, NCT03461458, NCT05011474) have not published their results for various reasons. One of the remaining two clinical trials (NCT02097862) evaluated the safety and efficacy of intradiscal injection of stromal vascular fraction (SVF) in combination with platelet rich plasma (PRP) in patients with degenerative disc disease[45]. There are AD-MSCs and growth factors in the SVF, but the adipocyte population has been depleted. Another phase I clinical trial (NCT02338271)

demonstrated the safety and tolerability of AD-MSCs combined with HA hydrogel therapy[46]. Although the data from these clinical trials preliminarily demonstrated the safety of autologous AD-MSCs in the treatment of DLBP, there are many shortcomings in these clinical trials, such as the limited number of samples, the lack of appropriate controls, and the lack of blinding. In order to further clarify the efficacy and safety of autologous AD-MSCs combined with HA hydrogel therapy, a large-sample phase II trial with matched controls is required. Based on previous promising findings, we design a double-blind, randomized controlled phase II clinical trial to evaluate the efficacy and safety of percutaneous intradiscal injection of autologous AD-MSCs combined with HA hydrogel scaffold in patients with DLBP.

METHODS AND ANALYSIS

Study design

This study is a randomized, dose-escalation, placebo-controlled, double-blind, single-center, Phase II clinical trial to evaluate the efficacy and safety of autologous AD-MSCs combined with HA hydrogel in the treatment of patients with DLBP. This trial will be conducted at the First Affiliated Hospital of Dalian Medical University in Dalian, Liaoning Province, China, and is expected to enroll 100 patients. After informed consent, patients will undergo relevant examinations, and only eligible patients can participate in clinical trials. All eligible subjects will undergo liposuction to obtain autologous ADMSCs, and then the ADMSCs and HA hydrogels will be formulated into different doses of stem cell mixtures. In the third week after liposuction, subjects will receive either different doses of stem cell mixtures or a placebo transplant, followed by a 24-month follow-up. The trial was registered in Chinese Clinical Trials Registry (http://www.chictr.org.cn) on April 4th, 2022 (Registration number ChiCTR2200058291). The detailed trial flow is described in figure 1.

Study objectives

There are two specific objectives of this Phase II clinical trial:

1. To evaluate the efficacy and safety of percutaneous intradiscal injections of ADMSCs combined with HA hydrogel in the treatment of patients with DLBP.
2. To determine the optimal therapeutic dose of ADMSCs.

Simple size and calculation

As a previous study indicated that after transplantation of 18 million allogeneic BM-MSCs, the mean visual analogue scale (VAS) score of IDD patients was reduced to 37.63±10.27 (10.3 reduction) after 24 months[47], we set this indicator at 10.6 reduction after 24 months of transplantation treatment. Furthermore, we assumed that α was 0.05, β was 0.1, and the dropout rate was 20%. Therefore, the calculated sample size for each group was 25, and the total sample size was 100 patients.

Eligibility Criteria

Patients with low back pain who obtained informed consent will only be allowed to participate in this clinical trial if they meet all the inclusion criteria and none of the exclusion criteria. The inclusion criteria is shown in Table 1, and the exclusion criteria is shown in Table 2.

Table 1. Inclusion criteria

Inclusion criteria
1.Patients who are male or female and whose age must be 18 years old or older.
2.Chronic LBP is accompanied by more than two (including two) clinical manifestations: increased pain when abdominal pressure increases such as cough and sneezing, increased pain when sedentary, forward bending or lifting heavy objects, difficult to relieve or unable to maintain the same posture, and pain relief when lying flat and resting.
3.Patients with LBP lasting 3 months or longer after conservative treatment.
4.VAS≥4.
5.ODI≥30%.
6.MRI shows that the CEPs of the lumbar IVD is Modic type I or II.
7.MRI shows that the L4-5 IVD is Pfirrmann grade 3, 4 or 5.
8.Discography of lumbar IVD(s) identified as degenerated by MRI show(s) that the patients have only one disc of L4-5 level with similar pain as usual.
9.Patients with ≥20% loss of lumbar disc height compared to normal adjacent discs based on X-ray assessment
10.Patients with no active infection (such as HBsAg, HIV, CMV and rubella virus).

LBP: Low back pain; VAS: Visual analog scale; ODI: Oswestry disability index; MRI: Magnetic resonance imaging; CEPs: Cartilage endplates; IVD: intervertebral disc; HBsAg: Hepatitis B surface antigen; HIV: Human immunodeficiency virus; CMV: Cytomegalovirus.

Table 2. Exclusion criteria

Exclusion criteria
1.Patients with spondylitis or vertebral fractures.
2.Surgery is required for patients with severe lumbar spinal stenosis or prolapse of the lumbar NP resulting in severe nerve compression and pain in the lower limbs.
3.Patients who have received any intradiscal injection procedure (eg, injection of corticosteroids, methylene blue, dextrose, or glucosamine and chondroitin sulfate) within the three months prior to receiving transplantation therapy.
4.Dynamic X-ray examination of the lumbar spine shows that the adjacent vertebral body slips > 3 mm or is angled > 15°.
5.Patients with severe osteoporosis with a BMD T value of -2.5 or lower on DEXA.
6.Pregnant or lactating women, or women who become pregnant within 24 months after receiving intervention.
7.Patients with mental illness or drug addictions or alcohol addictions or those incapable of understanding the purpose or methods of the study.
8.Patients with a history of various systemic diseases such as cancer, autoimmune disease, blood diseases, kidney diseases, or liver diseases.
9.Patients who are allergic to HA, contrast agents, or local anesthetics (eg, lidocaine, bupivacaine).
10.Patients who have previously used any other cell product and/or plan to participate in any other stem cell clinical trial during the 2-year follow-up period

NP: Nucleus pulposus; BMD: bone mineral density; DEXA: Dual-energy X-ray absorptiometry; HA: Hyaluronic acid.

AD-MSCs preparation and culture

All eligible patients have 150 ml of subcutaneous adipose tissue harvested by clinicians in the operating room through liposuction under local anesthesia 3 weeks before transplantation, and the patients are discharged after a 4-hour observation period. The harvested adipose tissue will be shipped to the cell factory. AD-MSCs will be obtained by washing, enzymatically dissociating, and centrifuging the adipose tissue using strict aseptic techniques, The cells will be plated on flasks and cultured at 37 °C in a humidified incubator under a 5% CO2 atmosphere. AD-MSCs used in this clinical trial will be obtained from cultured third-generation cells. These cells surface markers are positive for CD44, CD73, CD29 and negative for CD45 by flow cytometry, and the final products are tested to rule out the growth of aerobes, anaerobic bacteria and mycoplasma. In addition, cytogenetic analysis will be performed to rule out abnormal karyotypes. The cells are suspended at a concentration of 20×10^6 cells/ml of normal saline/vial, and these suspensions are transported to the operating room of the institute in a cold box at approximately 4 °C.

Preparation of HA hydrogel for cell delivery

The HA hydrogel scaffold chosen for this clinical trial is Tissefill, a transparent elastic gel composed of non-animal-derived HA derivatives. Cross-linked with butanediol diglycidyl ether, the gel resorbs nearly entirely in the body through enzymatic reactions. This HA hydrogel has been approved by South Korea's Ministry of Food and Drug Safety as a material for the delivery of cells and filling of tissue defects. Tissuefill is purchased from CHA Meditech Co., Ltd and is used to study the efficacy and safety of using autologous AD-MSCs combined with HA hydrogel in the treatment of DLBP. In a previous study, the optimal concentration of Tissuefill for injection into degenerated discs was determined and there was no cytotoxicity observed in the MSCs[48]. Therefore, in this clinical trial, we chose to use Tissuefill at a concentration of 1%.

Transplantation of AD-MSCs in combination with HA hydrogel

Based on discographic findings and confirmation of IDD with T2-weighted MRI, symptomatic discs are selected for transplantation. Under C-arm fluoroscopy, clinicians used spinal needles to percutaneously implant different doses of AD-MSCs with HA hydrogel (1% TIsefill) or saline into the IVD center via a standard posterolateral approach. The needle diameter of the spinal needle is 22G. After the transplantation, the patients take painkillers as needed for 3 days and restricted physical activity for 2 weeks.

Randomization and blinding

All the selected subjects are randomly assigned into the group according to the ratio of 1:1:1:1, and the statistician uses R software to generate a random sequence, a total of 4 groups with 25 cases in each group. In this experiment, the random envelope method is used for grouping concealment. Subjects who meet the eligibility criteria are assigned random numbers (001-100) in the order of enrollment, and then open the random envelopes with the corresponding numbers to obtain the corresponding grouping information.

This trial adopts a double-blind design. The drugs in the control group and the drugs

in the experimental groups are completely identical in appearance. During the transplantation treatment, both the patients and the clinicians performing the transplantation will be blinded. A statistician generates random numbers and corresponding drug codes, and then distributes and packs the drugs according to the codes, and prepares corresponding emergency letters. The computer program that generates the random numbers and the drug codes are kept as a blind bottom. Blind bases are in duplicate and kept in the trial responsible unit and agency respectively. A two-level blind design was adopted, the first level was the group corresponding to each case number, and the second level was the treatment corresponding to each group. The pharmacist dispenses the test drug and the placebo into a small sachet according to a single infusion dose. The sachets have the same shape and are opaque, and are marked with the serial number corresponding to the intervention category determined by the random number. Allocation tables recording serial numbers, random numbers, and group markers are kept in triplicate by the trial designer, the pharmaceutical company, and the pharmacy, respectively. Neither the trial designer nor the pharmacist participate in the trial.

Intervention

During the third week after liposuction, the subjects receive different doses of stem cell mixtures or placebos transplant. Subjects in the high-dose group receive a mixture that includes 1 ml of stem cell suspension (20×10^6 cells/disc), and 1 ml of Tissuefill (1%); Subjects in the mid-dose group receive a mixture that includes 0.5 ml of stem cell suspension (10×10^6 cells/disc), 0.5 ml of normal saline, and 1 ml of Tissuefill (1%); Subjects in the low-dose group receive a mixture that includes 0.25 ml of stem cell suspension (5×10^6 cells/disc), 0.75 ml of normal saline and 1 ml of Tissuefill (1%); Subjects in the control group receives 2 ml of normal saline injection. Because an earlier randomized controlled trial found no significant difference in the treatment of DLBP with HA alone and saline alone, a single HA solution control group is not necessary for this trial[47]. During the transplant process, neither the subjects nor the clinicians know the specific transplant treatment drug and doses.

Outcome evaluation

The primary endpoint of this trial is improvement in visual analogue scale (VAS) from baseline (prior to the transplantation) at each follow-up time point. Secondary endpoints include Oswestry disability index (ODI), Japanese orthopaedic association (JOA) scores, the Mos 36-item short form (SF-36), the Modic classification, Pfirrmann grade, height and segment range of motion of the IVD, vital signs (temperature, pulse, respiration, blood pressure), blood routine, liver and kidney function, immunological examination, urinalysis, and treatment emergent adverse events.The corresponding outcome measures and their time frames are listed in Table 3.

Table 3. Outcome measures and time frames

Outcome measures		Time frames
Primary outcome measures	VAS score	At baseline, 1week, 1, 3, 6, 12, 18 and 24 months
Secondary outcome measures	ODI score	At baseline, 1week, 1, 3, 6, 12, 18 and 24 months
	JOA scores system	
	SF-36 health survey score	
	The Modic changes of the IVD	At baseline, 6, 12, 24 months
	The Pfirrmann grade of the IVD	
	Disc height	
	Segment range of motion	
	Vital signs: temperature, pulse, respiration, blood pressure	At 1week, 1, 3, 6, 12, 18 and 24 months
	Blood routine	
	Liver and kidney function	
	Immunological examination	
	Urinalysis	
	Treatment emergent adverse event	

VAS: Visual analogue scale; ODI: Oswestry disability index; JOA: Japanese orthopaedic association; SF-36: the Mos 36-item short form; IVD: intervertebral disc.

Withdrawal

Discontinuation can occur as a result of death, serious adverse events (SAEs), other serious diseases limiting participation, or withdrawal by the subject requesting the study to be stopped. Those subjects who withdraw from the trial will have their withdrawal reasons and all observations recorded. New participants will not be recruited to replace withdrawn participants.

Adverse events

Adverse events (AEs) are defined as adverse medical events that occur after the patient signs informed consent until completion of the follow-up period. AEs include abnormal laboratory results, symptoms, or diseases. If the AE is confirmed to be a certain drug adverse reaction, it will be reported according to the adverse reaction reporting procedure of the research center. Once an AE occurs, the clinician will conduct necessary treatment according to the patient's condition and decide whether to suspend the clinical study. In terms of SAEs, clinicians should treat it as an emergency and will follow the principle of priority treatment. The researcher will report to the head of the center and the ethics committee of the research unit within 12 hours of the first learning, and report to the team leader within 24 hours or no later than the second working day. At the same time, researchers must handle the communication and aftermath of the subjects and their families.

Data collection

The data generated during the trial will be recorded in the original medical record and the case report form (CRF). To ensure that the data are entered accurately into the CRF, quality control personnel check the consistency of the CRF data with the original record. There are eight data collection points: baseline, 1 week, 1 month, 3 months, 6 months, 12 months, 18 months and 24 months. Research records will be submitted within 3 days of the completion of the data collection to the research leader for review and all data will be submitted within 10 days to the project leader. Next, the auditor will examine each original research record to ensure that the clinical trial data records are accurate, precise, and standardized. Data checks and entries will then be disposed of by the statistical data manager and analyzed by the statisticians.

Patient and public involvement

The patients and public were not involved in the design, or conduct, or reporting or dissemination plans of our research.

Statistical analysis

Statistical analysis will be performed using SPSS 22.0 software. All statistical tests are two-sided, the test level is α=0.05, and the confidence interval is 95% confidence level. The primary focus of the data analysis is to determine the effect of any treatment at each follow-up point (1 week and 1, 3, 6, 12, 18, and 24 months post-transplant). In addition, linear mixed models are applied to assess differences in treatment effect between groups at each follow-up point. In addition, linear mixed models are applied to assess differences in treatment effect between groups (20×10^6 cells/disc, 10×10^6cells/disc, and 5×10^6cells/disc) at each follow-up point. According to the type of variables and data distribution, *t* test, analysis of variance or nonparametric test is used for measurement data, and $\chi2$ test is used for enumeration data. The safety evaluation is mainly based on descriptive statistical analysis, listing adverse events, serious adverse events, the number of times leading to adverse events, and calculating the incidence.

DISCUSSION

This Phase II clinical trial will answer two key questions for patients and the scientific community. First, whether autologous AD-MSCs combined with HA hydrogel is safe and effective in the treatment of DLBP. Second, what is the optimal dose of AD-MSCs for the treatment of DLBP. Although many studies have been conducted on BM-MSCs, and the results of phase III clinical trials have also demonstrated their efficacy, the invasiveness of obtaining BM-MSCs makes many patients hesitant. The emergence of AD-MSCs perfectly fills this gap and makes stem cell therapy more acceptable to patients, which will greatly promote stem cell therapy to the clinic. However, stem cell therapy also faces challenges. First of all, in vitro studies have shown that the harsh microenvironment inside the degenerated IVD will affect the biological behaviors of stem cells, such as viability, proliferation and differentiation, and then affect the therapeutic effect of stem cell therapy[49,50]. Secondly, the clinical research on AD-MSCs is still in a relatively immature stage, and the specific therapeutic mechanism of stem

cells is still lacking in-depth understanding. Therefore, it is necessary for us to carry out more clinical trials to further explore. Although there are relatively few clinical trials for DLBP treatment, AD-MSCs are still a promising type of MSCs. The successful implementation of this clinical trial will provide data support for subsequent phase III clinical trials, and will also significantly promote the clinical application of AD-MSCs. DLBP patients are about to usher in a new era of ADMSCs therapy.

Author Contributions ZHL contributed to the conception and design of the study. JZ wrote the first draft of the manuscript. WTZ, TZS, and MY supervised the manuscript. All authors contributed to manuscript revision and approved the submitted version.

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Competing interests All authors declare to have no competing interest concerning this work.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

ETHICS AND DISSEMINATION The study protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University and registered in Chinese Clinical Trial Registry. Dissemination of the results will be presented at a conference and in peer-reviewed publications.

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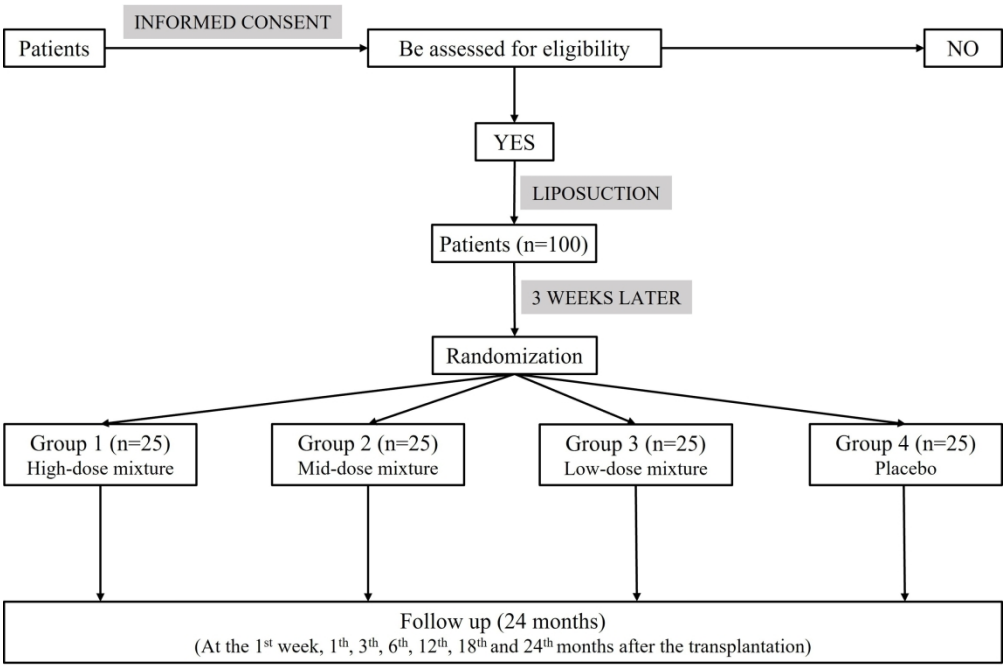
Figure legend

Fig. 1 Study flow diagram



Study flow diagram

252x167mm (300 x 300 DPI)

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Autologous cultured adipose derived mesenchymal stem cells combined with hyaluronic acid hydrogel in the treatment of discogenic low back pain: a study protocol for a phase II randomized controlled trial

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Autologous cultured adipose derived mesenchymal stem cells combined with hyaluronic acid hydrogel in the treatment of discogenic low back pain: a study protocol for a phase II randomized controlled trial

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ABSTRACT

Introduction: Discogenic low back pain (DLBP) is a common disease, and its occurrence is closely related to intervertebral disc degeneration (IDD). At present, none of the traditional treatment methods can repair the degenerated intervertebral disc (IVD). The emergence of stem cell therapy makes it possible to repair and regenerate IVDs tissue, among which adipose derived mesenchymal stem cells (ADMSCs) transplantation therapy has become a hot spot of current research. Therefore, this trial aimed to investigate the safety and efficacy of using autologous cultured ADMSCs combined with hyaluronic acid (HA) hydrogel in the treatment of DLBP.

Methods and analysis: This study is a randomized, dose-escalation, placebo-controlled, double-blind, single-center, Phase II clinical trial to evaluate the efficacy and safety of autologous cultured ADMSCs combined with HA hydrogel in the treatment of patients with DLBP. The 100 eligible patients will be randomly divided into 3 experimental groups with different doses and 1 placebo control group in a ratio of 1:1:1:1. All patients will undergo liposuction to obtain ADMSCs, followed by autologous cultured ADMSCs mixtures or placebo transplantation after three weeks. The patients will be followed up to 24 months after the transplant. The primary endpoint of this trial is the visual analogue scale (VAS). Secondary endpoints include Oswestry disability index (ODI), Japanese orthopaedic association (JOA) scores, the Mos 36-item short form (SF-36), the Modic classification, Pfirrmann grade, height and segment range of motion of the IVD, vital signs (temperature, pulse, respiration, blood pressure), blood routine, liver and kidney function, immunological examination, urinalysis, and treatment emergent adverse events.

Ethics and dissemination: The study protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University and registered in Chinese Clinical Trial Registry. Dissemination of the results will be presented at a conference and in peer-reviewed publications.

Trial registration: ChiCTR2200058291

KEYWORDS

Protocols & guidelines; Back pain; Spine

Strengths and limitations of this study

► This study is a randomized, placebo-controlled, double-blind trial, so bias will be minimized.

► The subjects of this trial are recruited from only one research center, and the sample size is not large enough.

► The subjects of this trial are patients with DLBP with single disc segment degeneration, which would limit applicability to the general population.

INTRODUCTION

Low back pain (LBP) is a common health concern worldwide. According to some studies, the point prevalence of LBP is 11.9%, and the one-month prevalence is 23.3%. LBP is the main reason for years lived with disability and places a heavy burden on patients and society[1-3]. Discogenic low back pain (DLBP) is a common source of LBP, with an overall prevalence of 26%~42%, and in the younger population, this rate reaches more than 80%[4,5]. DLBP gradually evolves from internal intervertebral disc (IVD) diseases such as inflammation, deformation and annulus fibrosus (AF) injury, and its key pathological process is IVD degeneration (IDD)[6,7]. The clinical manifestations of patients with DLBP are recurrent LBP, especially when sitting for a long time, bending over or coughing, but there is often no positive feature of nerve root damage during physical examination. Although the pathological mechanism of DLBP has not been fully understood, it is widely believed that the sensory nerve fibers from the outer layer of the IVD grow into the interior through the fissure, and the production of proinflammatory cytokines in the degenerated IVD increases, resulting in back pain in patients[8-10]. In addition, abnormal lumbar disc activity due to long-term mechanical injury may accelerate the progression of DLBP[11,12]. The diagnosis of DLBP requires

a combination of imaging findings and long-term recurrent LBP. Clinically, magnetic resonance imaging (MRI) is an essential tool for evaluating IVD pathology, and its signal characteristics reflect the degree of IVD aging or degeneration[7]. Many patients with DLBP are found to have decreased signal intensity in the IVD on MRI T2-weighted imaging, which is the so-called "black" IVD, and the sagittal view shows that the posterior border of the IVD has a small, round, limited area of high signal intensity[13,14]. However, the relationship between the degree of disc degeneration on imaging and the severity of pain remains unclear, so it is necessary to rely on CT-guided discography for further diagnosis. Discography is currently the only "gold standard" test for diagnosing DLBP. Following injection of the contrast agent into the disc, patients will experience similar pain responses, but the adjacent disc will not[14]. Based on discography, DLBP may be categorized as AF ruptured LBP and cartilage endplates (CEPs) ruptured LBP, and this classification method has clinical and theoretical support[15]. Due to the varying severity of clinical manifestations in patients with DBLP, step-by-step therapy is often used in the treatment. Traditional treatment methods include conservative, interventional, and surgical treatments[15]. Most patients tend to opt for conservative treatments due to mild symptoms and a short course of the disease, including bed rest, physiotherapy with microwave or infrared, oral painkillers, and functional exercises. Interventional treatments, such as epidural injections and percutaneous intradiscal therapies, are generally performed if conservative treatments fail[16-18]. With severe symptoms or ineffective conservative and interventional treatments, surgical treatments are often recommended. The most commonly used surgical procedures are interbody fusion and artificial disc replacement[18]. However, conservative treatments have limited efficacy, and interventional treatments for pain relief should be further evaluated. Although surgical treatments can effectively relieve pain, they may cause complications such as infection, nerve damage, large blood vessel damage and adjacent segment degeneration due to improper operation or care, which will further damage the body of patients[19,20]. Additionally, the best way to treat DLBP would be to slow down or even reverse the process of IDD, but neither conservative treatments, interventional treatments nor surgical treatments can do anything about it.

Traditional treatments are in a dilemma, and a new treatment is urgently needed to induce repair of degenerated disc tissue.

The IVD is composed of an outer AF, a nucleus pulposus (NP) in the middle, and CEPs at the upper and lower ends. NP is the main structure of IVD, which is mainly composed of NP cells and extracellular matrix (ECM). NP cells are cartilage-like cells. The main components of ECM are COL II, proteoglycans and other matrix proteins[21]. IDD mainly occurs in NP. With the degradation of ECM and the loss of proteoglycans, these changes will reduce the structural integrity of IVD and eventually damage its function[22]. The recent deepening of the understanding of IDD has promoted the research of biological therapy, among which stem cell therapy stands out and becomes the current research hotspot. In the past few decades, human stem cell therapy indications have involved many different fields, including neurological diseases, cardiovascular diseases, diabetes, blood diseases and cancer, with exciting results[23-26]. Given the broad application prospects of stem cell therapy in regenerative medicine, people have begun to explore the application in spinal degenerative diseases. DLBP is a common spinal degenerative disease, and the key to its treatment is to repair the degenerated disc tissue and reduce the discomfort of patients. The transplanted stem cells are capable of self-replication, renewal, and multi-directional differentiation, which can differentiate into chondrocytes to replace the lost NP cells, as well as promoting the formation of ECM by co-culturing with NP cells[27-29]. In addition, stem cells have immunomodulatory effects and can secrete various cytokines to improve the microenvironment inside the IVD, promote the repair of degenerated IVD tissue, and relieve the pain of patients[27-29]. Stem cells for the treatment of IDD come from a wide range of sources, including bone marrow mesenchymal stem cells (BMSCs) and adipose derived MSCs (ADMSCs), and a few stem cell types derived from human umbilical cord MSCs (HUCMSCs), pluripotent stem cells (PSCs), NP-derived stem cells (NPSCs) and other sources[29,30]. Among the many types of stem cells, BMSCs are currently the most studied, and their safety and efficacy in the treatment of IDD have been verified in clinical trials, bringing the dawn of stem cell clinical treatment for IDD[31,32]. However, due to the cumbersome and invasive process of obtaining BMSCs,

its clinical application is limited to a certain extent[33,34]. With the deepening of research, it has been found that ADMSCs have similar chondrogenic differentiation potential compared with BMSCs. More importantly, ADMSCs have the advantages of easy to obtain in large quantities, lower incidence of donor site and higher proliferation potential, so they may be an ideal source of stem cells for the treatment of DLBP[33,35].

During stem cell therapy, potential complications such as leakage and osteophyte formation may occur at the infusion site, and the implanted stem cells are subject to high mechanical loads in the disc, which may reduce the viability or function of the stem cells. In order to solve these problems, various scaffolds have been designed as carriers for delivering stem cells, among which hydrogel scaffolds are more commonly used[36-38]. Among various biomaterials for the production of hydrogel scaffolds, HA and its derivatives have been extensively studied. HA is a naturally occurring glycosaminoglycan that is involved in vital processes such as cell proliferation, migration, angiogenesis, and tissue growth. Biocompatibility, biodegradability, processability, and tunable mechanical properties of HA contribute to its clinical appeal[39-41]. Therefore, the transplantation of ADMSCs combined with HA hydrogel has become a hot research topic.

The efficacy of ADMSCs in the treatment of DLBP has been verified in animal models[42-44]. In order to further verify whether stem cell therapy is also safe and effective in humans, it is necessary to conduct clinical trials. Currently, six clinical trials of ADMSCs in the treatment of IDD are registered on the ClinicalTrials.gov website, and four of them (NCT01643681, NCT03461458, NCT05011474, NCT02529566) have not published their results for various reasons. One of the remaining two clinical trials (NCT02097862) evaluated the safety and efficacy of intradiscal injection of stromal vascular fraction (SVF) in combination with platelet rich plasma (PRP) in patients with degenerative disc disease[45]. There are ADMSCs and growth factors in the SVF, but the adipocyte population has been depleted. Another phase I clinical trial (NCT02338271) demonstrated the safety and tolerability of ADMSCs combined with HA hydrogel therapy[46].

Safety is an important consideration in conducting clinical trials. Since the IVD

contains cartilage tissue, researchers also pay attention to the progress made by ADMSCs in treating articular cartilage injuries. The safety of ADMSCs in repairing articular cartilage has been demonstrated in clinical trials conducted in the United States[47,48], China[49,50], South Korea[51], Italy[52], France[53] and Australia[54]. The progress made by ADMSCs in the treatment of cartilage injury has significantly increased our confidence in their application to the treatment of DLBP.

Although the data from these clinical trials preliminarily demonstrated the safety of autologous cultured ADMSCs in the treatment of DLBP, there are many shortcomings in these clinical trials, such as the limited number of samples, the lack of appropriate controls, and the lack of blinding. In order to further clarify the efficacy and safety of autologous cultured ADMSCs combined with HA hydrogel therapy, a large-sample phase II trial with matched controls is required. Based on previous promising findings, we design a double-blind, randomized controlled phase II clinical trial to evaluate the efficacy and safety of percutaneous intradiscal injection of autologous cultured ADMSCs combined with HA hydrogel scaffold in patients with DLBP.

METHODS AND ANALYSIS

Study design

This study is a randomized, dose-escalation, placebo-controlled, double-blind, single-center, Phase II clinical trial to evaluate the efficacy and safety of autologous cultured ADMSCs combined with HA hydrogel in the treatment of patients with DLBP. This trial will be conducted at the First Affiliated Hospital of Dalian Medical University in Dalian, Liaoning Province, China, and is expected to enroll 100 patients. After informed consent, patients will undergo relevant examinations, and only eligible patients can participate in clinical trials. All eligible subjects will undergo liposuction to obtain autologous cultured ADMSCs, and then the ADMSCs and HA hydrogels will be formulated into different doses of stem cell mixtures. In the third week after liposuction, subjects will receive either different doses of stem cell mixtures or a placebo transplant, followed by a 24-month follow-up. The trial was registered in

Chinese Clinical Trials Registry (http://www.chictr.org.cn) on April 4th, 2022 (Registration number ChiCTR2200058291). The detailed trial flow is described in figure 1.

Study objectives

There are two specific objectives of this Phase II clinical trial:

1. To evaluate the efficacy and safety of percutaneous intradiscal injections of ADMSCs combined with HA hydrogel in the treatment of patients with DLBP.
2. To determine the optimal therapeutic dose of ADMSCs.

Simple size and calculation

As a previous study indicated that after transplantation of 18 million allogeneic BMSCs, the mean visual analogue scale (VAS) score of IDD patients was reduced to 37.63±10.27 (10.3 reduction) after 24 months[55], we set this indicator at 10.6 reduction after 24 months of transplantation treatment. Furthermore, we assumed that α was 0.05, β was 0.1, and the dropout rate was 20%. Therefore, the calculated sample size for each group was 25, and the total sample size was 100 patients.

Eligibility Criteria

Patients with LBP who obtained informed consent will only be allowed to participate in this clinical trial if they meet all the inclusion criteria and none of the exclusion criteria. The inclusion criteria is shown in Table 1, and the exclusion criteria is shown in Table 2.

Table 1. Inclusion criteria

Inclusion criteria
1.Patients who are male or female and whose age must be 18 years old or older.
2.Chronic LBP is accompanied by more than two (including two) clinical manifestations: increased pain when abdominal pressure increases such as cough and sneezing, increased pain when sedentary, forward bending or lifting heavy objects, difficult to relieve or unable to maintain the same posture, and pain relief when lying flat and resting.
3.Patients with LBP lasting 1 year or longer after conservative treatment.
4.VAS≥4.
5.ODI≥30%.
6.MRI shows that the CEPs of the lumbar IVD is Modic type I or II.

7.MRI shows that the L4-5 IVD is Pfirrmann grade 3, 4 or 5.
8.Discography of lumbar IVD(s) identified as degenerated by MRI show(s) that the patients have only one disc of L4-5 level with similar pain as usual.
9.Patients with ≥20% loss of lumbar disc height compared to normal adjacent discs based on X-ray assessment
10.Patients with no active infection (such as HBsAg, HIV, CMV and rubella virus).

LBP: Low back pain; VAS: Visual analog scale; ODI: Oswestry disability index; MRI: Magnetic resonance imaging; CEPs: Cartilage endplates; IVD: intervertebral disc; HBsAg: Hepatitis B surface antigen; HIV: Human immunodeficiency virus; CMV: Cytomegalovirus.

Table 2. Exclusion criteria

Exclusion criteria
1.Patients with spondylitis or vertebral fractures.
2.Surgery is required for patients with severe lumbar spinal stenosis or prolapse of the lumbar NP resulting in severe nerve compression and pain in the lower limbs.
3.Patients who have received any intradiscal injection procedure (eg, injection of corticosteroids, methylene blue, dextrose, or glucosamine and chondroitin sulfate) within 1 year prior to receiving transplantation therapy.
4.Dynamic X-ray examination of the lumbar spine shows that the adjacent vertebral body slips > 3 mm or is angled > 15°.
5.Patients with severe osteoporosis with a BMD T value of -2.5 or lower on DEXA.
6.Pregnant or lactating women, or women who become pregnant within 24 months after receiving intervention.
7.Patients with mental illness or drug addictions or alcohol addictions or those incapable of understanding the purpose or methods of the study.
8.Patients with a history of various systemic diseases such as cancer, autoimmune disease, blood diseases, kidney diseases, or liver diseases.
9.Patients who are allergic to HA, contrast agents, or local anesthetics (eg, lidocaine, bupivacaine).
10.Patients who have previously used any other cell product and/or plan to participate in any other stem cell clinical trial during the 2-year follow-up period

NP: Nucleus pulposus; BMD: bone mineral density; DEXA: Dual-energy X-ray absorptiometry; HA: Hyaluronic acid.

Recriument

Participants will be recruited from three sources. First, the potentially eligible hospitalized patients diagnosed with DLBP will be approached and recommended for enrollment in this study. Second, physicians will generate a list of patients with DLBP who have not undergone surgery from the hospital's electronic records. Researchers or physicians will contact these patients by phone and recommend them to participate in the study. Third, physicians will post study flyers at the outpatient department and the official website for patients diagnosed with DLBP at other hospitals, and if they are interested in this study, we will initiate the screening process.

ADMSCs preparation and culture

All eligible patients have 150 ml of subcutaneous adipose tissue harvested by clinicians in the operating room through liposuction under local anesthesia 3 weeks before transplantation, and the patients are discharged after a 4-hour observation period. The harvested adipose tissue will be shipped to the cell factory. ADMSCs will be obtained by washing, enzymatically dissociating, and centrifuging the adipose tissue using strict aseptic techniques, The cells will be plated on flasks and cultured at 37 °C in a humidified incubator under a 5% CO2 atmosphere. ADMSCs used in this clinical trial will be obtained from cultured third-generation cells. These cells surface markers are positive for CD44, CD73, CD29 and negative for CD45 by flow cytometry, and the final products are tested to rule out the growth of aerobes, anaerobic bacteria and mycoplasma. In addition, cytogenetic analysis will be performed to rule out abnormal karyotypes. The cells are suspended at a concentration of 20×10^6 cells/ml of normal saline/vial, and these suspensions are transported to the operating room of the institute in a cold box at approximately 4 °C.

Preparation of HA hydrogel for cell delivery

The molecular weight of HA plays an important role in modulating the inflammation in LBP during disc repair. It is generally believed that high molecular weight HA inhibits the activation of pro-inflammatory cytokines and matrix-degrading enzymes, whereas low molecular weight HA promotes inflammatory and tissue remodeling[56-58]. The implantable HA hydrogel in this trial is prepared by mixing 1% FCH-200 with fibrin solution and incubating it at room temperature for 15 minutes. FCH-200 is a high molecular weight HA (molecular weight: 1800-2200 kDa), purchased from Kikkoman Bio Chemifa Co., Ltd. A previous study has shown that this HA promotes the aggregation of ADMSCs and induce their differentiation towards cartilage[59]. Therefore, the scaffold material selected for this clinical trial is HA hydrogel with FCH-200 as the gel matrix.

Transplantation of ADMSCs in combination with HA hydrogel

Based on discographic findings and confirmation of IDD with T2-weighted MRI, symptomatic discs are selected for transplantation. Under C-arm fluoroscopy, clinicians used spinal needles to percutaneously implant different doses of ADMSCs with the HA hydrogel or saline into the IVD center via a standard posterolateral approach. The needle diameter of the spinal needle is 22G. After the transplantation, the patients will be asked to restrict physical activity for 2 weeks.

Randomization and blinding

All the selected subjects are randomly assigned into the group according to the ratio of 1:1:1:1, and the statistician uses R software to generate a random sequence, a total of 4 groups with 25 cases in each group. In this experiment, the random envelope method is used for grouping concealment. Subjects who meet the eligibility criteria are assigned random numbers (001-100) in the order of enrollment, and then open the random envelopes with the corresponding numbers to obtain the corresponding grouping information.

This trial adopts a double-blind design. The injections of the control group and the injections of the experimental groups are completely identical in appearance. During the transplantation treatment, both the patients and the clinicians performing the transplantation will be blinded. A statistician generates random numbers and corresponding drug codes, and then distributes and packs the drugs according to the codes, and prepares corresponding emergency letters. The computer program that generates the random numbers and the drug codes are kept as a blind bottom. Blind bases are in duplicate and kept in the trial responsible unit and agency respectively. A two-level blind design was adopted, the first level was the group corresponding to each case number, and the second level was the treatment corresponding to each group. The pharmacist dispenses the test drug and the placebo into a small sachet according to a single infusion dose. The sachets have the same shape and are opaque, and are marked with the serial number corresponding to the intervention category determined by the random number. Allocation tables recording serial numbers, random numbers, and group markers are kept in triplicate by the trial designer, the pharmaceutical company,

and the pharmacy, respectively. Neither the trial designer nor the pharmacist participate in the trial.

Intervention

During the third week after liposuction, the subjects receive different doses of stem cell mixtures or placebos transplant. Subjects in the high-dose group receive a mixture that includes 1 ml of stem cell suspension (20×10^6 cells/disc), and 1 ml of HA hydrogel; Subjects in the mid-dose group receive a mixture that includes 0.5 ml of stem cell suspension (10×10^6 cells/disc), 0.5 ml of normal saline, and 1 ml of HA hydrogel; Subjects in the low-dose group receive a mixture that includes 0.25 ml of stem cell suspension (5×10^6 cells/disc), 0.75 ml of normal saline and 1 ml of HA hydrogel; Subjects in the control group receives 2 ml of normal saline injection. Because the purpose of this study is to investigate the efficacy and safety of stem cells combined with HA hydrogel in the treatment of DLBP, there is no separate HA hydrogel control group. During the transplant process, neither the subjects nor the clinicians know the specific transplant treatment drug and doses.

Outcome evaluation

The primary endpoint of this trial is improvement in visual analogue scale (VAS) from baseline (prior to the transplantation) at each follow-up time point. Secondary endpoints include Oswestry disability index (ODI), Japanese orthopaedic association (JOA) scores, the Mos 36-item short form (SF-36), the Modic classification, Pfirrmann grade, height and segment range of motion of the IVD, vital signs (temperature, pulse, respiration, blood pressure), blood routine, liver and kidney function, immunological examination, urinalysis, and treatment emergent adverse events. Each follow-up time point will be conducted by telephone and outpatient contacts. Patients or their families will be reminded by phone the day before the follow-ups. The corresponding outcome measures and their time frames are listed in Table 3.

Table 3. Outcome measures and time frames

Outcome measures			Time frames
Primary measures	outcome	VAS score	At baseline, 1week, 1, 3, 6, 12, 18 and 24 months
Secondary measures	outcome	ODI score	At baseline, 1week, 1, 3, 6, 12, 18 and 24 months
		JOA scores system	
		SF-36 health survey score	
		The Modic changes of the IVD	At baseline, 6, 12, 24 months
		The Pfirrmann grade of the IVD	
		Disc height	
		Segment range of motion	
		Vital signs: temperature, pulse, respiration, blood pressure	At 1week, 1, 3, 6, 12, 18 and 24 months
		Blood routine	
		Liver and kidney function	
		Immunological examination	
		Urinalysis	
		Treatment emergent adverse event	

VAS: Visual analogue scale; ODI: Oswestry disability index; JOA: Japanese orthopaedic association; SF-36: the Mos 36-item short form; IVD: intervertebral disc.

Adverse events

Adverse events (AE) are defined as adverse medical events that occur after the patient signs informed consent until completion of the follow-up period. AE include abnormal laboratory results, symptoms, or diseases. If the AE is confirmed to be a treatment related adverse reaction, it will be reported according to the adverse reaction reporting procedure of the research center. Once an AE occurs, the clinician will conduct necessary treatment according to the patient's condition and decide whether to suspend the clinical study. Serious AE (SAE) refer to life-threatening medical events such as paralysis, tumors, serious infections and even death of patients during clinical trials. In terms of SAE, clinicians should treat it as an emergency and will follow the principle of priority treatment. The researcher will report to the head of the center and the ethics committee of the research unit within 12 hours of the first learning, and report to the team leader within 24 hours or no later than the second working day. At the same time, researchers must handle the communication and aftermath of the subjects and their families.

Withdrawal

Discontinuation can occur as a result of SAE such as paralysis, tumors, serious

infections or even death, other serious diseases limiting participation, or withdrawal by the subject requesting the study to be stopped. Those subjects who withdraw from the trial will have their withdrawal reasons and all observations recorded. New participants will not be recruited to replace withdrawn participants.

Data and safety monitoring

The members of the Data and Safety Monitoring Board (DSMB) are independent of the trial investigators and have no competing interests. Clinical safety and efficacy data collected at the time intervals specified in the protocol will be reviewed and evaluated by the DSMB. The DSMB will be notified if the safety data threshold exceeds a predefined threshold. Additionally, the DSMB will conduct an interim analysis of all AE occurrences every six months during the course of the study. All investigators and monitors will have access to the electronic trial data during the data collection period; after completion of the trial, the data will also be accessible to statisticians. All of the data will be provided to the DSMB.

Data collection

The data generated during the trial will be recorded in the original medical record and the case report form (CRF). To ensure that the data are entered accurately into the CRF, quality control personnel check the consistency of the CRF data with the original record. There are eight data collection points: baseline, 1 week, 1 month, 3 months, 6 months, 12 months, 18 months and 24 months. Research records will be submitted within 3 days of the completion of the data collection to the research leader for review and all data will be submitted within 10 days to the project leader. Next, the auditor will examine each original research record to ensure that the clinical trial data records are accurate, precise, and standardized. Data checks and entries will then be disposed of by the statistical data manager and analyzed by the statisticians.

Patient and public involvement

The patients and public were not involved in the design, or conduct, or reporting or

dissemination plans of our research.

Statistical analysis

Statistical analysis will be performed using SPSS 22.0 software. All statistical tests are two-sided, the test level is α=0.05, and the confidence interval is 95% confidence level. The primary focus of the data analysis is to determine the effect of any treatment at each follow-up point (1 week and 1, 3, 6, 12, 18, and 24 months post-transplant). In addition, linear mixed models are applied to assess differences in treatment effect between groups at each follow-up point. In addition, linear mixed models are applied to assess differences in treatment effect between groups (20×10^6 cells/disc, 10×10^6cells/disc, and 5×10^6cells/disc) at each follow-up point. According to the type of variables and data distribution, *t* test, analysis of variance or nonparametric test is used for measurement data, and $\chi2$ test is used for enumeration data. The safety evaluation is mainly based on descriptive statistical analysis, listing adverse events, serious adverse events, the number of times leading to adverse events, and calculating the incidence.

Rules for unblinding

Unblinding is carried out according to normal procedures if no subject pregnancy or other emergency occurs during the course of the trial. First, after checking the CRF and signature, the grouping of subjects will be clarified through first-level unblinding for statistical analysis. Second, after the statistical analysis, the corresponding control and experimental groups will be identified through secondary unblinding to evaluate the efficacy of stem cell injections. If there is an emergency, emergency unblinding can only be performed if the investigator must have information on the treatment assignment of subjects in emergency. The investigator will unblind according to the treatment information provided by the subject in the emergency letter, and then complete the unblinding record form and note it on the CRF. After the trial, the number, reason, scope and time of emergency unblinding should be described and analyzed as a reference for the evaluation of efficacy and safety.

DISCUSSION

This Phase II clinical trial will answer two key questions for patients and the scientific community. First, whether autologous cultured ADMSCs combined with HA hydrogel is safe and effective in the treatment of DLBP. Second, what is the optimal dose of ADMSCs for the treatment of DLBP. Although many studies have been conducted on BMSCs, and the results of phase III clinical trials have also demonstrated their efficacy, the invasiveness of obtaining BMSCs makes many patients hesitant. The emergence of ADMSCs perfectly fills this gap and makes stem cell therapy more acceptable to patients, which will greatly promote stem cell therapy to the clinic. However, stem cell therapy also faces challenges. First of all, in vitro studies have shown that the harsh microenvironment inside the degenerated IVD will affect the biological behaviors of stem cells, such as viability, proliferation and differentiation, and then affect the therapeutic effect of stem cell therapy[60,61]. Secondly, the clinical research on ADMSCs is still in a relatively immature stage, and the specific therapeutic mechanism of stem cells is still lacking in-depth understanding. Therefore, it is necessary for us to carry out more clinical trials to further explore. Although there are relatively few clinical trials for DLBP treatment, ADMSCs are still a promising type of MSCs. The successful implementation of this clinical trial will provide data support for subsequent phase III clinical trials, and will also significantly promote the clinical application of ADMSCs. DLBP patients are about to usher in a new era of ADMSCs therapy.

ETHICS AND DISSEMINATION

The study protocol has been approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University and registered in Chinese Clinical Trial Registry. Dissemination of the results will be presented at a conference and in peer-reviewed publications. An insurance company will provide insurance coverage for damages

emerging from the trial.

Protocol amendments

All protocol amendments will be evaluated by the Ethics Committee and the Chinese National Medical Products Administration, following the principles of Good Clinical Practice and national legislation. All modifications of the study protocol will be communicated by updating the trial registry at Chinese Clinical Trials Registry (http://www.chictr.org.cn).

Availability of data and materials

All the data in the trial will be available for anyone who wants to access the data following publication.

Dissemination policy

Output from this study will include journal publications, conference presentations and community reporting. Output will not identify participants.

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Author Contributions ZHL contributed to the conception and design of the study. JZ wrote the first draft of the manuscript. WTZ, TZS, and MY supervised the manuscript. All authors contributed to manuscript revision and approved the submitted version.

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Competing interests All authors declare to have no competing interest concerning this work.

Patient consent for publication Not required.

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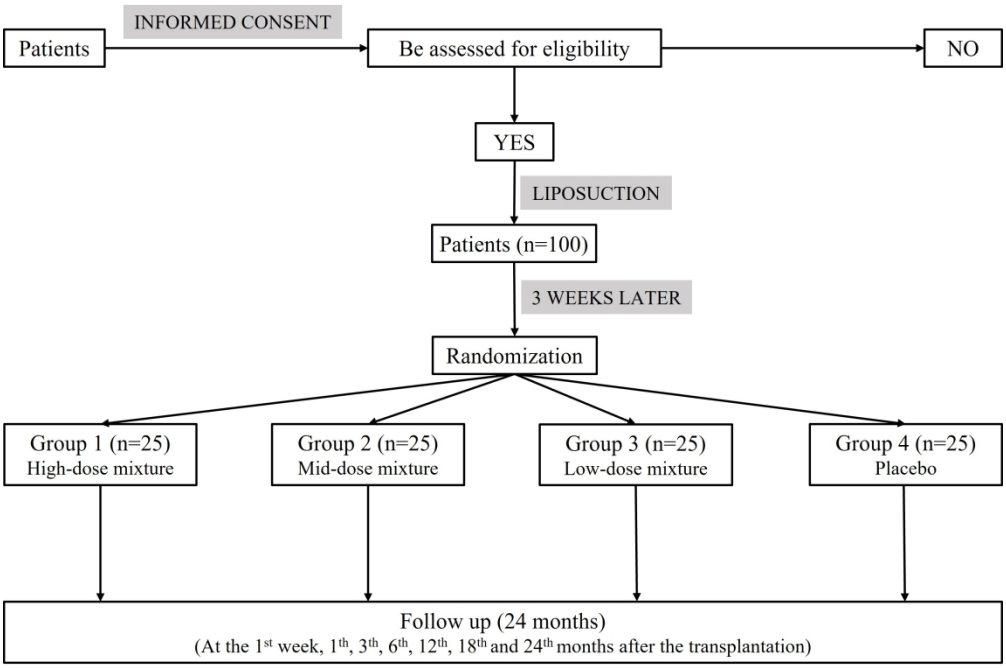
Figure legend

Fig. 1 Study flow diagram



Study flow diagram

252x167mm (330 x 330 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	7-8
Protocol version	3	Date and version identifier	2022.04.04 4/2022, page 7-8
Funding	4	Sources and types of financial, material, and other support	17
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 17
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	17
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-7
	6b	Explanation for choice of comparators	12
Objectives	7	Specific objectives or hypotheses	8
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7-8, 11-12
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7-8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8-9, Table 1, Table 2
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	13-14
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10-11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12-13, Table 3
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7-8, Figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	15	Strategies for achieving adequate participant enrolment to creach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11-12
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11-12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11-12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11-12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	15
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-13, Table 3
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Not applicable
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Not applicable
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13-14
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13-14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	13-14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16-17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	17

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7-8
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not appliable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	16-17
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	17
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	17
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available on request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not appliable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.