PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Zhang, Jing; Sun, Tianze; Zhang, Wentao; Yang, Ming; Li, Zhonghai

VERSION 1 – REVIEW

REVIEWER	Bodor, Marko University of California San Francisco, Neurosurgery
REVIEW RETURNED	19-May-2022

GENERAL COMMENTS	Abstract: you should insert the word “cultured” after “autologous” to indicate that you will be culturing the cells. Introduction: Recommend ending the first sentence after “23.3%” then starting the next sentence as follows: “LBP is the main reason for years lived with disability and places a heavy burden on patients and society.” The sentence “Based on discography..” has some abbreviations, “AF” and “CEP”, which need to be defined. Delete “resulting in affect the treatment effect.” Inclusion criteria – if you are including patients with LBP for 3 months or longer, there is a good chance some of them are going to get better spontaneously, thus making it harder for your active group to be better than placebo. Why not make your inclusion criteria LBP for at least 1 year or longer? This should ensure that the patients problems are truly chronic and the likelihood of spontaneous improvement would be lower. So are you only including patients with L4-5 disc problems? Exclusion criteria – are you sure that injected corticosteroids, at least long-acting corticosteroids, have been cleared by the disc within 3 months of injection? They may remain much longer. Perhaps you should change the 3 months to 1 year. Intervention: The relative difference in doses, 20, 10 and 5 million cells is a relatively small range of 4-fold. How did you choose this range? Perhaps you should consider doses of 1 million, 10 million and 100 million cells? Regarding “painkillers” – these should not be NSAIDs or aspirin, which inhibit prostaglandin mediated vasodilation and have been shown recently to inhibit healing of acute injuries and increase the chances of an acute injury becoming chronic. Regarding “drugs in the control group”, it would be better to use the term “injectate in the control group.” Regarding “a certain drug adverse reaction” – this would be better termed as a “treatment related adverse reaction” After all, you are

	not injecting “a drug” but a cellular treatment. “SAE” needs to be defined. Discussion and throughout the text: after the word “autologous” the word “cultured” should be inserted to make it clear that these are “autologous cultured AD-MSCs”

REVIEWER	Srivastava, Akshay National Institute of Pharmaceutical Education and Research Ahmedabad
REVIEW RETURNED	26-Jul-2022

GENERAL COMMENTS	The study is nicely designed with clarity of various controls and eligibility criteria. The following minor issues need to be addressed: 1. Grammatical errors in punctuations and spell mistake should be corrected. 2. Authors used AD-MSCs and ADMSCs at various place. Make it consistent throughout the manuscript, preferably use ADMSCs. 3. Page no. 12, Line no. 53-59. Author said that HA control group is not required because no significant difference were found earlier trial in the the treatment of DLBP with HA alone. Author should mention the molecular weight of HA used in previous study and in the study conducted by author. Molecular weight of HA play an important role in modulating the inflammation in LBP (Appl. Sci. 2020, 10(18), 6257; https://doi.org/10.3390/app10186257).

REVIEWER	Manchikanti, Laxmaiah Pain Management Center of Paducah
REVIEW RETURNED	29-Aug-2022

GENERAL COMMENTS	This is a well-conceived design and executes protocol. Completion of this trial as planned will provide insights into disc degeneration and the role of mesenchymal stem cells. It is particularly of importance as adipose-derived mesenchymal stem cells are not permitted to be used in the United States. Presentation of more and more evidence and demonstration of the safety may lead to a path for limited approvals in the United States.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Marko Bodor, Napa Medical Research Foundation

Comments to the Author:

Abstract: you should insert the word “cultured” after “autologous” to indicate that you will be culturing the cells.

Response: We appreciate the reviewer's suggestion and have inserted the word "cultured" after "autologous" in the abstract section.

Details: 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.

Introduction: Recommend ending the first sentence after “23.3%” then starting the next sentence as follows: “LBP is the main reason for years lived with disability and places a heavy burden on patients and society.”

Response: We greatly appreciate this recommendation and have reworked this sentence in the introduction section.

Details: 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 economic burden on patients and society.

The sentence “Based on discography.” has some abbreviations, “AF” and “CEP”, which need to be defined.

Response: We completely agree with this valuable suggestion by the reviewer. According to the reviewer’s suggestion, we have added the definitions of AF and CEPs.

Details:

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).

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.

Delete “resulting in affect the treatment effect.”

Response: We appreciate this suggestion and have deleted "resulting in affect the treatment effect."

Details: 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.

Inclusion criteria – if you are including patients with LBP for 3 months or longer, there is a good chance some of them are going to get better spontaneously, thus making it harder for your active group to be better than placebo. Why not make your inclusion criteria LBP for at least 1 year or longer? This should ensure that the patients problems are truly chronic and the likelihood of spontaneous improvement would be lower.

Response: Thank you for your valuable comment. We completely agree with this valuable suggestion by the reviewer. Since patients with DLBP requiring treatment primarily present with chronic LBP, which is defined as LBP for over 12 weeks, we included patients with LBP for 3 months or longer. Indeed, as you said, if the inclusion criteria for LBP are set at 1 year or longer, it is better to ensure that the patient's problems are truly chronic, and the likelihood of spontaneous improvement would be lower. Therefore, we have adopted this recommendation and the inclusion criteria have been revised.

Details: Patients with LBP lasting 1 year or longer after conservative treatment.

So are you only including patients with L4-5 disc problems?

Response: We appreciate the reviewer's comment. There are two main reasons why we decided to only include patients with L4-5 disc problems in this study. Firstly, the positive discography of DLBP patients is mainly concentrated in L4-5 and L5-S1. To improve the accuracy of this study, we included only L4-5 in order to reduce the influence of confounding factors. Secondly, as one of the largest general hospitals in China, our research unit has an enormous number of patients suffering from DLBP, so there should be no problem with the number of subjects.

Details: 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.

Exclusion criteria – are you sure that injected corticosteroids, at least long-acting corticosteroids, have been cleared by the disc within 3 months of injection? They may remain much longer. Perhaps you should change the 3 months to 1 year.

Response: We greatly appreciate and endorse the reviewer's suggestion. In this study, it may be better if the time after intradiscal corticosteroid injection is changed from 3 months to 1 year in order to avoid the influence of corticosteroids. Therefore, we modified this exclusion criterion.

Details: 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.

Intervention: The relative difference in doses, 20, 10 and 5 million cells is a relatively small range of 4-fold. How did you choose this range? Perhaps you should consider doses of 1 million, 10 million and 100 million cells?

Response: Thank you for your valuable comment. We completely agree with this valuable suggestion by the reviewer. Before conducting our study, we referred to the results of a previous phase I clinical trial that used ADMSCs [46]. The 10 patients in this phase I trail underwent a single intradiscal injection of combined HA derivative and ADMSCs at a dose of 2×10^7 cells/disc (n = 5) or 4×10^7 cells/disc (n = 5). One-year follow-up results showed significant improvements in VAS, ODI, and SF-36 scores in both groups, and there was no significant difference between them. Therefore, under the premise of ensuring that the dose of implanted ADMSCs has a good repair effect on the IVD, we try to

choose a lower dose of stem cells. Additionally, stem cells have been used in many clinical trials, including BMSCs, at doses between millions and tens of millions [31,32,45,55]. To further clarify the optimal therapeutic dose of ADMSCs, we believe that a relatively small 4-fold range is appropriate.

Details:

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.

31. Noriega DC, Ardura F, Hernández-Ramajo R, et al. Intervertebral Disc Repair by Allogeneic Mesenchymal Bone Marrow Cells: A Randomized Controlled Trial. Transplantation. 2017;101(8):1945-1951.

32. Noriega DC, Ardura F, Hernández-Ramajo R, et al. Treatment of Degenerative Disc Disease With Allogeneic Mesenchymal Stem Cells: Long-term Follow-up Results. Transplantation. 2021;105(2):e25-e27.

45. 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.

46. 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(1):262.

55. Amirdelfan K, Bae H, McJunkin T, et al. Allogeneic mesenchymal precursor cells treatment for chronic low back pain associated with degenerative disc disease: a prospective randomized, placebo-controlled 36-month study of safety and efficacy. Spine J. 2021;21(2):212-230.

Regarding “painkillers” – these should not be NSAIDs or aspirin, which inhibit prostaglandin mediated vasodilation and have been shown recently to inhibit healing of acute injuries and increase the chances of an acute injury becoming chronic.

Response: We are grateful for the suggestion. As mentioned by the reviewer, taking painkillers may increase the chance of acute injury becoming chronic, which may unnecessarily interfere with the results of this study. Therefore, we decided not to allow the patients to take painkillers after the surgery.

Regarding “drugs in the control group”, it would be better to use the term “injectate in the control group.”

Response: We completely agree with this valuable suggestion by the reviewer. According to the reviewer’s suggestion, we have replaced "drugs" with "injections."

Details: The injections of the control group and the injections of the experimental groups are completely identical in appearance.

Regarding “a certain drug adverse reaction” – this would be better termed as a “treatment related adverse reaction” After all, you are not injecting “a drug” but a cellular treatment.

Response: We appreciate the reviewer's suggestion and have replaced “a certain drug adverse reaction” with “treatment related adverse reaction.”

Details: 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.

“SAE” needs to be defined.

Response: Thanks to the reviewer for making the correction. We have supplemented the definition of SAE and adjusted the order of the "Adverse events" and "Withdraw" sections to make the article more logical.

Details:

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.

Discussion and throughout the text: after the word “autologous” the word “cultured” should be inserted to make it clear that these are “autologous cultured AD-MSCs”

Response: Thank you for your valuable comment. We completely agree with this valuable suggestion by the reviewer. According to the reviewer's suggestion, we have inserted the word "cultured" after "autologous" in nine places of the full text.

Reviewer: 2

Dr. Akshay Srivastava, National Institute of Pharmaceutical Education and Research Ahmedabad

Comments to the Author:

The study is nicely designed with clarity of various controls and eligibility criteria. The following minor issues need to be addressed:

1. Grammatical errors in punctuations and spell mistake should be corrected.

Response: We apologize for the grammatical and spelling errors in the original manuscript. As suggested by the reviewer, the language presentation has been improved by correcting and polishing the grammar and spelling of the full text.

2. Authors used AD-MSCs and ADMSCs at various place. Make it consistent throughout the manuscript, preferably use ADMSCs.

Response: We completely agree with this valuable suggestion by the reviewer. According to the reviewer's suggestion, we have changed all the "AD-MSCs" in the full text to "ADMSCs". In addition, in order to unify the abbreviation format for stem cells, "BM-MSCs" and "HUC-MSCs" have also been replaced by "BMSCs" and "HUCMSCs" respectively.

3. Page no. 12, Line no. 53-59. Author said that HA control group is not required because no significant difference were found earlier trial in the the treatment of DLBP with HA alone. Author should mention the molecular weight of HA used in previous study and in the study conducted by author. Molecular weight of HA play an important role in modulating the inflammation in LBP (Appl. Sci. 2020, 10(18), 6257; https://doi.org/10.3390/app10186257).

Response: We completely agree with this valuable suggestion by the reviewer. We apologize for not mentioning the molecular weight of hyaluronic acid from various studies. As you pointed out, the molecular weight of HA plays an important role in modulating the inflammation in LBP [56-58]. Unfortunately, despite a comprehensive literature review, we have not been able to identify the exact molecular weight of the Tissuefill product and the HA product used alone in the earlier trial, and therefore cannot determine whether the molecular weight is consistent between them. To avoid such problems, we will replace the original Tissuefill product with HA hydrogel containing FCH-200 as the gel matrix. FCH-200 is a high molecular weight HA (molecular weight: 1800-2200 kDa), which will be purchased from Kikkoman Bio Chemifa Co., Ltd. This HA hydrogel has been shown to well promote the aggregation of ADMSCs and induce their differentiation towards cartilage, so it is a good scaffold material for ADMSCs [59]. It will be used to study the safety and efficacy of autologous cultured ADMSCs in combination with HA hydrogel in treating DLBP.

Details:

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.

56. Kazezian Z, Joyce K, Pandit A. The role of hyaluronic acid in intervertebral disc regeneration. Applied Sciences. 2020;10(18):6257.

57. Isa ILM, Srivastava A, Tiernan D, et al. Hyaluronic Acid Based Hydrogels Attenuate Inflammatory Receptors and Neurotrophins in Interleukin-1β Induced Inflammation Model of Nucleus Pulposus Cells. Biomacromolecules. 2015;16(6):1714-1725.

58. Kazezian Z, Sakai D, Pandit A. Hyaluronic Acid Microgels Modulate Inflammation and Key Matrix Molecules toward a Regenerative Signature in the Injured Annulus Fibrosus. Advanced Biosystems. 2017;1(10):1700077.

59. Wu S-C, Chen C-H, Wang J-Y, et al. Hyaluronan size alters chondrogenesis of adipose-derived stem cells via the CD44/ERK/SOX-9 pathway. Acta Biomaterialia. 2018;66:224-237.

Reviewer: 3

Dr. Laxmaiah Manchikanti, Pain Management Center of Paducah

Comments to the Author:

This is a well-conceived design and executes protocol. Completion of this trial as planned will provide insights into disc degeneration and the role of mesenchymal stem cells.

It is particularly of importance as adipose-derived mesenchymal stem cells are not permitted to be used in the United States.

Presentation of more and more evidence and demonstration of the safety may lead to a path for limited approvals in the United States.

Response:

We are very grateful for Professor Manchikanti's comments. We completely agree with his valuable suggestion. As we all know, in view of the developed medical environment and perfect medical system in the United States, the approval requirements for clinical trials in the United States may be higher than other countries. In recent years, clinical trials of stem cell therapy for degenerative disc disease have been carried out extensively around the world. Adipose-derived mesenchymal stem cells have gradually attracted the attention of researchers because of their unique advantages. There are currently six clinical trials of ADMSCs in the treatment of degenerative disc disease registered on Clinicaltrails.gov, three of which are conducted in the United States (NCT02097862, NCT03461458, NCT02529566) and the remaining three in South Korea (NCT02338271, NCT05011474, NCT01643681). Among these trials, only one phase I trial was completed in the United States, and it used stromal vascular fractions (SVF) enriched with ADMSCs rather than pure ADMSCs [45]. Clearly, it is difficult to conduct relevant clinical trials in the United States directly using ADMSCs. Fortunately, among the three studies conducted in Korea, there is a phase I clinical trial that truly preliminarily verified the safety and tolerability of ADMSCs combined with HA derivatives in the treatment of DLBP [46]. This result provides an important theoretical support for us to use ADMSCs combined with HA hydrogel to carry out the next clinical trial.

As the IVD contains cartilage tissue, we concentrated not only on the ability of ADMSCs to repair the disc, but also on their research progress with regards to articular cartilage repair. In the only two clinical trials in the United States with published outcome papers, the researchers used autologous SVF [47] and autologous adipose tissue [48] instead of ADMSCs to treat knee osteoarthritis, respectively. Although they are not pure ADMSCs, these results can indirectly reflect the safety and efficacy of ADMSCs in repairing articular cartilage. In addition to the United States, relevant clinical trials have also been conducted in countries such as China [49,50], South Korea [51], Italy [52], France [53] and Australia [54]. The results of these trials fully demonstrated the safety of ADMSCs in the treatment of articular cartilage injury. The progress made by ADMSCs in the treatment of cartilage injury has significantly increased our confidence in their application to the treatment of DLBP.

We believe that with the deepening of research, there will be more and more clinical trial data to prove the safety of ADMSCs therapy, and the application of ADMSCs may gradually be recognized and approved in the United States.

Details:

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.

45. 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.

46. 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(1):262.

47. Garza JR, Campbell RE, Tjoumakaris FP, et al. Clinical Efficacy of Intra-articular Mesenchymal Stromal Cells for the Treatment of Knee Osteoarthritis: A Double-Blinded Prospective Randomized Controlled Clinical Trial. The American journal of sports medicine. 2020;48(3):588-598.

48. Jones IA, Wilson M, Togashi R, et al. A randomized, controlled study to evaluate the efficacy of intra-articular, autologous adipose tissue injections for the treatment of mild-to-moderate knee osteoarthritis compared to hyaluronic acid: a study protocol. BMC musculoskeletal disorders. 2018;19(1):383.

49. Qiao Z, Tang J, Yue B, et al. Human adipose-derived mesenchymal progenitor cells plus microfracture and hyaluronic acid for cartilage repair: a Phase IIa trial. Regenerative medicine. 2020;15(1):1193-1214.

50. Lu L, Dai C, Du H, et al. Intra-articular injections of allogeneic human adipose-derived mesenchymal progenitor cells in patients with symptomatic bilateral knee osteoarthritis: a Phase I pilot study. Regenerative medicine. 2020;15(5):1625-1636.

51. Lee WS, Kim HJ, Kim KI, et al. Intra-Articular Injection of Autologous Adipose Tissue-Derived Mesenchymal Stem Cells for the Treatment of Knee Osteoarthritis: A Phase IIb, Randomized, Placebo-Controlled Clinical Trial. Stem cells translational medicine. 2019;8(6):504-511.

52. Peretti GM, Ulivi M, De Girolamo L, et al. Evaluation of the use of autologous micro-fragmented adipose tissue in the treatment of knee osteoarthritis: preliminary results of a randomized controlled trial. Journal of biological regulators and homeostatic agents. 2018;32(6 Suppl. 1):193-199.

53. Pers YM, Rackwitz L, Ferreira R, et al. Adipose Mesenchymal Stromal Cell-Based Therapy for Severe Osteoarthritis of the Knee: A Phase I Dose-Escalation Trial. Stem cells translational medicine. 2016;5(7):847-856.

54. Freitag J, Ford J, Bates D, et al. Adipose derived mesenchymal stem cell therapy in the treatment of isolated knee chondral lesions: design of a randomised controlled pilot study comparing arthroscopic microfracture versus arthroscopic microfracture combined with postoperative mesenchymal stem cell injections. BMJ open. 2015;5(12):e009332.

VERSION 2 – REVIEW

REVIEWER	Bodor, Marko University of California San Francisco, Neurosurgery
REVIEW RETURNED	02-Oct-2022

GENERAL COMMENTS	All of my questions and concerns have been addressed. We are looking forward to the results of the study.